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PRINCIPAL INVESTIGATOR: John Colanduoni, Ph.D.

CONTRACTING ORGANIZATION: Arista Biologicals, Incorporated
Bethlehem, Pennsylvania 90501

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13. ABSTRACT *(Maximum 200 words)*

Arista Biologicals, Inc. proposes to develop a series of assays to detect infectious disease pathogens and toxins on a series of rapid nitrocellulose membrane based assays utilizing colloidal gold and fluorescent dyes in both a unitized and multi-analyte format capable of semi-quantitation with a simple, inexpensive reader similar to a home diabetic glucose meter. We have formatted and initially tested 6 of the pathogens in the unitized format and have developed working tests for both dengue and hanta viruses. We plan to continue our investigation of the use of fluorescent dyes side by side with visible dyes, as the initial work has shown as at least comparable results and indicates that with a UV reader we may well find the enhanced sensitivity that we originally predicted. We have completed the exploratory work and initial testing of a reader system for enhanced readability and quantification and plan on continuing those efforts in the Phase II segment of the project. All of these efforts will enhance the detection capabilities of rapid assays for both military forward deployment and civilian point of care applications

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FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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( ) In conducting research using animals, the investigator(s) adhered to the 'Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

( )For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 32 CFR 219 and 45 CFR 46.

( )In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

Principal Investigator's Signature

7/9/97
Date

DAMD17-97-C-7027

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ATTACHMENTS:

- ATTACHMENT A - SAMPLES OF READER GRAPHING
- ATTACHMENT B - REQUEST TO AMEND CONTRACT
- ATTACHMENT C - SAMPLE PACKAGE INSERT
- ATTACHMENT D - INFORMATION ON (+) DENGUE SERA
- ATTACHMENT E - METHODOLOGY PROCEDURES

INTRODUCTION

Arista Biologicals, Inc. (ABI), as the prime contractor, and Majesco Biologicals, Inc., as an affiliated company, propose to develop a series of rapid membrane based tests to screen medical and environmental samples for the presence of toxins, bacterial antigens, and/or antibodies and to develop a simple reader system capable if increasing sensitivity and specificity, enabling quantification, and removing objectivity in visual reading of such membrane based tests. The aim is to address the problems experienced with diagnostic immunoassay testing in the field and/or by laymen and less technically personnel.

There has been a stereotyping of these tests as being somewhat inferior to more classic methods of diagnostic testing. Many early generation tests were inferior in quality and prematurely released before proper controls were available. This led many in the medical field to believe these tests were not capable of the same accuracy as ELISA, PCR or biosensors. It is our belief that by using the basic immunochromatographic membrane based assays it is possible to develop tests equal, or superior, in accuracy and dependability as these other types of assays. Most of the other confirmatory methods require more expensive equipment, sophisticated electronic technology and long multi-step procedures, drawbacks not associated with our assay format.

These benefits are important features for a test that is intended to be performed by users in the field with limited technical training. The system's design will make it much less expensive, much faster and easier to perform by the end user than other technologies including ELISA, PCR and bio-sensor systems. In that ELISA is the test most frequently compared to our rapid membrane format, it should be pointed out that the differences are significant:

ELISA	**Membrane**
Multi-step	One step
Minimum of 30-60 mins.	3-5 minutes
Primarily instrument based	non-instrumented
labile stability	totally stable for 2yrs
cost effective if multiple specimens	same cost single or multiple
much higher tech know-how	anyone can run
single analyte	potential for multiple analytes

Currently, ABI utilizes colloidal gold particles as the conjugate in the above described chromatographic system. In the past, we have successfully produced a variety of assays for infectious disease testing. As part of our Phase I project, we investigated the use of fluorescent probes to replace or enhance the colloidal gold system, either attempting to attach the fluorescent probes directly to the detector antibody or proteins and/or attaching the fluorescent probes to the colloidal gold detector protein conjugates. The use of any such fluorescent probe modification should enable us to increase the sensitivity of these membrane based assays as much as one thousand fold. This adaptation can increase the use of this technology not only as a screening test but as a confirmatory assay and will allow that the system will still be visually readable in situations when the optional reader with its enhanced sensitivity and objectivity features are not required.

As a second goal of the Phase I SBIR, ABI has modified this immunochromatographic assay into a multi-analyte test format that will make it more applicable in both the military and civilian sectors such as in detection of environmental contaminants, and screening to differentially diagnose infectious diseases.

The potential benefits associated with these assays and reader systems have garnered the interest of both environmental and infectious disease sections of the DOD as well as that of several well placed commercial partners. These assays have particular application for military forward deployment and point of care use in Third World countries where the assay characteristics of simple, one step, non-instrumented, non-technical expertise, 3-5 minute result assays are essential. This presents a creditable scenario for the Dual Use role of these assays. The exceptional stability of the components/reagents of these products under any conditions is an additional factor in their desirability in all markets.

Arista staff have developed assays, in house, for individual clients whose interests cover most areas of diagnostics, such as veterinarian, environmental, drug testing, bacterial and viral pathogens, hormones, specific antibody, etc.. ABI staff also train other companies in the production of these types of tests for either standard products or products developed specifically for the client. Our staff has up to ten years experience in this area of the business. The assays developed can be used for serum, plasma, whole blood, urine, feces, airborne particles, dirt, etc. Several of the assays have been tested in trials, including very successful field trials of plague, anthrax, and leishmania.

THE PROJECT:

This project has three basic areas that are being investigated simultaneously:

1. The first task is to develop a rapid, hand-held immunoassay system capable of being used in the field with state of the art sensitivity utilizing fluorescent probes combines with colored particles in an effort to enhance detection capability and allow visualization by an ultraviolet light source.

2. The second task is to develop an immunoassay system capable of being read with a small hand-held reader that will perform quantification, providing a permanent written record. This system will also allow for visual determination of sensitivity utilizing a densitometer.

3. The third task in this project will be the ability to perform several assays on a single device in order to reduce the number of individual tests being run by the operator. These multi-analyte tests would differentiate between multiple agents that are suspected of being present.

4. As a component of this project, work has been done to optimize the basic format for the detection of Hanta virus and Dengue virus with the ability to develop a test for relevant antibody detection.

GENERAL METHODOLOGY:

In this section we provide some detail on specific materials/ reagents used within our tests as well as some narrative on methodology for preparing actual test strips in our format. A more complete description of production procedures and methodology is contained in a separate, 40 page document submitted as an attachment to this report. Note that unless there is some variation in procedures, methods, or reagents, this information will be presented only in this section and not for each of the individual projects.

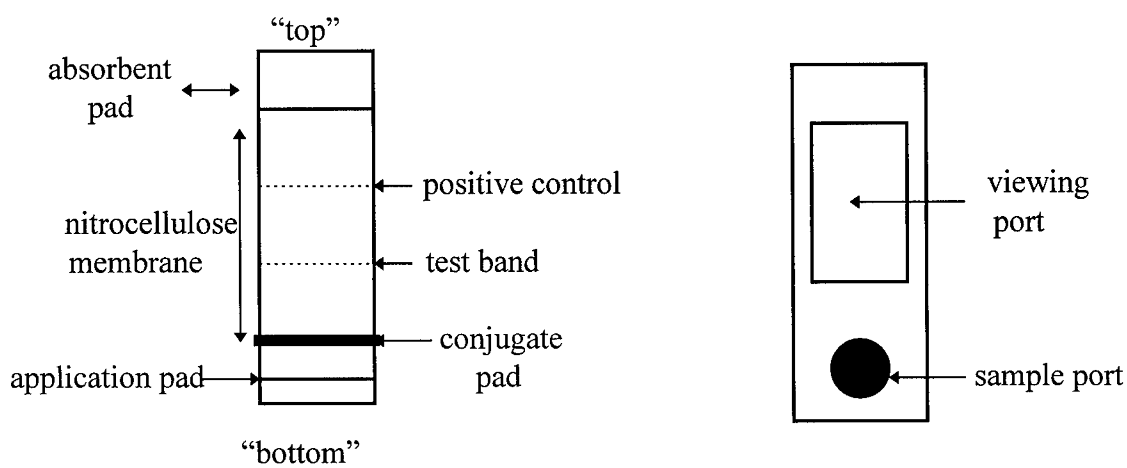
- **buffer optimization** - Assay buffer is incorporated into the sample pad. We start with standard pad for serum and buffer and work out from there. This pad is soaked with 200 mM Sodium Borate pH 9.3. 0.1% Triton X-100, 0.5% PVA. We will normally adjust this formulation based on whether we see false positive results. For example, we will raise the pH up to as high as 11 and increase the Triton X-100 to up to 1% or add up to 1% SDS. If false positives are still a problem we will add an assay buffer such as 1% BSA, 0.1% Sodium Azide, pH 7.4 50 mM Sodium Phosphate and dilute the sample with that diluent directly on the assay pad, thereby diluting out the serum as much as

75%. We can also add any specific reagent to remove problems such as E. coli extract, goat serum, other surfactants, etc. This is normally only necessary for antibody assays where the antigen we spray on the membrane contains contaminants which the sera sometimes reacts with. The above described diluent may also be used as a general diluent to increase flow rate of viscous or lipemic samples which when run neat sometimes take over 10 minutes to run.

- **membranes (pore size) and colloidal gold (particle sizes)** - Our previous experiences with other strip colloidal gold systems have taught us that a pore size of approximately 15 microns is the best for both flow rate and reactivity. If larger pore sizes are used the flow rate is usually too fast for reactions to take place at low sensitivities and if smaller pore sizes are used a test is difficult to finish in 5 minutes. We generally use the SR (15μ) series of nitrocellulose membranes from Millipore. We did, however, look at 20 micron pore size nitrocellulose for the fluorescent latex assays used in this study because the latex is about 10 times larger than the colloidal gold. As to colloidal gold particle size, previous experience in gold assays has shown us that a 40 nm particle is the best for the strip assays. Smaller particles give less signal because of how the gold scatters light and larger particles tend to migrate slower, generate a purple blue color, and are difficult to work with (stability). The latex had to be approximately 250 nm in order to be able to centrifuge and process the conjugates. This is due to its density being approximately 9 times less that of the gold.

- **production methodology** - The first step is to take 30 cm plastic cards (3 inches by 30 cm) and peel off the cover of the double stick adhesive that is present 1 inch lengthwise in the center of the card. The nitrocellulose membrane is then laminated onto this section prior to dispensing the capture reagent. The capture reagent is then dispensed on the card using the Biodot Quantijet Dispenser at a volume of approximately 1 microliter per cm at a predetermined concentration (typically at 1 to 4 milligrams per ml). The card is dried at 37 degrees for one hour, then normally blocked with a BSA sucrose (20% sucrose) mixture, and then re-dried as before. While the cards are drying the conjugate pad material, polyester, is also pretreated with PVA and surfactant to make it hydrophilic and allow conjugate to dry and later re-hydrate. The absorbent pad of cellulose acetate is also soaked with the assay buffer (normally made up of sodium borate, BSA and or PVA, and surfactant(s)). These materials are also air dried and can be stored for long periods of time. The colloidal gold or latex conjugate is then dispensed on the conjugate pad material at the predetermined concentration and volume and is then also air dried at 37 degrees Celsius. After all of the materials are dried, the absorbent pads, conjugate pads, and sprayed and blocked cards are assembled by removing the rest of the covers of the double stick tape on each card (overlapping the conjugate pad on the bottom of the nitrocellulose and the absorbent pad which itself overlaps with the conjugate pad). Untreated cellulose acetate is then placed and overlapped with the upper portion of the nitrocellulose. The cards are then slit into strips of the desired width, (this width is 4 cm for the device used in this project) and stored with desiccant while awaiting final assembly. The strips are later placed into the plastic housings and closed and pouched into foil pouches along with desiccant and droppers. Pouches are labeled with lot # and name of test. Sensitivity and specificity is tested for each lot to assure appropriate antigen or antibody levels of detection.

(Figure 1) **Standard test format**



absorbent pad - cellulose acetate
sample pad - cellulose acetate

conjugate pad - polyester
conjugate - protein A colloidal gold (PAG)

- **format methodology** - the sample is added to the sample well (**Figure 1**):
    - The sample reconstitutes the assay buffer. The filter materials also acts to filter out any large particles that might contaminate the sample. The next material that the sample comes in contact with is the conjugate pad (containing the identifying antibody). The conjugate pad is also buffer treated so as to promote reconstitution of the conjugate and maintain immunological activity of the dyed or colored conjugate.
    - The test sample then can react with the conjugate if the test sample contains antigen specific for the antibody conjugate.
    - This mixture then migrates onto the nitrocellulose membrane. The nitrocellulose membrane section has two horizontal lines of reagent material sprayed onto it in a single analyte format and multiple horizontal lines in the multi-analyte format.

- When a capture protein or related material is sprayed onto the nitrocellulose, it becomes non-covalently attached to the membrane because of the membrane's high affinity for proteins and other hydrophobic characteristic materials. The lower band (or bands in multi-analyte) has (have) the capture antibody sprayed onto it in a sandwich assay or the specific antigen sprayed onto it in an antibody capture assay. The conjugate has the detector antibody attached to it in a sandwich assay and the conjugate has the "anti-antibody" reagent attached to it in the antibody capture assay. If the conjugate has the correct antigen attached to it so that the capture protein recognizes it, the colored or dyed particle will then attach to the lower line of the strip creating a visual or visualizable horizontal line.
- Any conjugate that migrates beyond the first horizontal test band will then attach to the second band since the second band is always an anti-conjugate material. For example, in the case of a monoclonal antibody conjugate an anti-mouse IgG antibody is used as a positive control capture reagent. Therefore, two horizontal bands are seen for a positive reaction and only one, the higher band, is observed in a negative test result.

- If for some reason the test is invalid, there will be no band observed and the sample will need to be re-tested or investigated for the cause of the invalid result.

GENERAL INFORMATION:

- **separation of IgM and IgG** - We have performed preliminary experiments to separate out reactions to IgM and IgG simultaneously on a single strip for the antibody assays. We decided to use the test for dengue as the prototype. We did this by placing two test bands on the strip. One was goat anti-human IgM and the other anti-human IgG. Dengue antigen was conjugated to the colloidal gold particle. Previous experience with Epstein-Barr assays had shown us that this is difficult to accomplish with good success, however in that assay we were working with lysate type antigen and we thought that the recombinant antigen would work better in this manner. We have seen some separation of signal, but it definitely needs more work, which we will continue in Phase II.

- **avidin/biotin use** - A streptavidin/biotin common reagent system was evaluated but was not found to enhance sensitivity and was not even as sensitive as direct conjugation of primary antibodies. We had similar experiences earlier on in other projects. This is probably because our assay is very similar to a homogeneous assay and is not sequential in nature where streptavidin biotin works best. We have also had problems with this system with individuals who were on large doses of vitamins where it interfered with biotin binding because of similarities in structure with biotin of some vitamins.

- **use of F-1 and plague antigens** - We are preparing to immunize with plague antigens. We would need to commit additional goats but do not see spending money till we see if we are going to continue, in that there is limited use for our own commercial applications. We also need more antigen for making affinity columns. Antisera will be useless without antigen for affinity purification. Antibody material we have from the Army now is good enough, as far as activity levels, for a feasibility study.

PROJECT PROGRESS REPORT:

1. Fluorescent Probes

Strategy 1 - The fluorescent probes were evaluated using several methods in our attempt to increase sensitivity of the strip assays. Because of the nature of the experiments these trials used hundreds of milligrams of antibodies. The target reagents (ricin, SEB, etc.) are available in only limited quantities, and therefore hCG reagent (in abundant, inexpensive supply) was used as the model. Initial studies used direct conjugation of the fluorescent tags to the monoclonal antibody replacing the colloidal gold conjugates normally used in the strips. Rhodamine, Texas red, and fluorescein were used as the fluorescent conjugate. The conjugation **methodology** was as follows:

> The initial experiments conjugating the fluorescent probes directly to the antibodies were all performed using isothiocyanate derivatives of the organic compounds. Isothiocyanate will couple directly to proteins without requiring other chemicals. We conjugated in pH 7.4 PBS at 10 mg of probe to 1 mg of protein. They were allowed to stir at 4 degrees centigrade in the dark overnight and dialyzed three times at 1000 fold excess dialysis buffer. The dialysis buffer was pH 7.4 PBS.

The highest color intensity visually observed when ultraviolet light was placed on the strips was observed when using fluorescein. Fluorescein was attached using FITC as the fluorescent tag and optimal conjugation observed at approximately 10 mg of FITC to 1 mg of antibody at pH 7.4 in PBS. However, when placed in the standard hCG strip it appeared to be only as sensitive as the normal colloidal gold assay to approximately 1 ng/ml. We also needed to use approximately ten times more antibody conjugate per test using about 10 ug of antibody per test compared to a colloidal gold antibody conjugate.

Strategy 2 - The next strategy taken was to add the fluorescent probe to the colloidal gold antibody conjugate directly hoping that we would get a higher FITC to particle ratio than conjugating to individual antibodies since the antibody gold conjugate contains many antibody molecules per particle. The gold is approximately 40 nm in size and coated with many smaller antibody molecules. When tested, this showed almost no fluorescence observed at even 100 ng/ml levels of hCG. Several levels of hapten conjugation were attempted from 1 to 100 mg of label per mg of conjugate without any positive results. This may be due to interactions with the more hydrophobic gold or contaminants in the gold preparations.

Strategy 3 - The final, and most successful method, was found to be with using latex fluorescent beads and coating the hCG antibody to these particles in the following manner. Three fluorescent types of beads were purchased with approximately 300 nm particle size. The 300 nm was chosen because at smaller size particle ranges it is very hard to separate unconjugated antibody from the antibody bead molecules since the density of the latex particles is not much higher than water. This compares to the gold particles which have an approximate tenfold higher density than water. Larger latex particles tend to clog the nitrocellulose membranes being used in this project since they are close to the membrane pore sizes used (typically 3 to 15 microns) and also tend to settle in solution when being applied.

Fluorescein, Texas Red, and Rhodamine containing latex particles were purchased. Both standard polystyrene and carboxylated particles were purchased for antibody conjugation optimization. Conjugation **methodology** for the latex particles was as follows:

> Using approximately 300 nm polystyrene latex particles from Bangs Laboratories and using FITC, Texas Red, rhodamine dyed latex, the latex particles were coated in the following manner. The latex particles were diluted to 0.1% solids/volume in pH 6.8 100 mM Sodium Phosphate. Antibody was added at 1 mg/10 ml of diluted latex particles. The mixture was rocked overnight at room temperature. The latex was washed three times by centrifugation and re-suspension into pH 7.4 PBS in a tabletop centrifuge at 3000 rpm for 15 minutes and then re-suspended by vortex. The final concentration was 1 ml per 10 ml of starting material making the final concentration 1% solid/volume. The conjugates were used directly in the assays in both a liquid and dried form on the strip.
>
> Latex particles carboxylated were conjugated in the following manner. The latex was diluted into PBS to 0.1% solid/volume and solid carbodiimide added to the mixture and rocked for 30 minutes for activation. The mixture was then centrifuged for 15 minutes at 3000 rpm and re-suspended twice into pH 7.4 PBS. Antibody was added as above and washed as above. The latex thus produced seemed identical to that in the first procedure so that the final decision was to use the polystyrene latex and the passive absorption method. We had hoped the 2nd method would allow for an increase of binding or more activity due to covalent attachment instead of passive absorption.

In this latex phase of the project, it was found that again the fluorescein type materials showed the highest fluorescence. We expected the carboxylated particles to work better but, suprisingly, this was not the case. Normally, covalent attachment forms a nonreversible binding making a more stable conjugate and a more active conjugate than passively absorbed conjugates using the nude polystyrene particles. This is thought to occur since the covalent attachment allows for more free movement of the antibody on the surface of the particle. The conjugates were then placed on strips coated previously with hCG, varying the hCG in the sample and the volume of conjugate. The passively absorbed material was found to be about 5 times more active. The sensitivity again was found to be approximately 1 ng/ml although now we needed approximately 1 microgram of antibody per test.

Although initially we were disappointed to see that we did not detect the expected increase in sensitivity using these fluorescent probes as compared to the colloidal gold, discussing is with several peers in the industry allowed us to arrive at the **following conclusion**. Normally, when using fluorescent probes in immunoassays, the fluorescence is not visually read but rather is read by instrumentation. The signal may be in the non-visual range towards the ultraviolet range of the spectrum. We most likely need to use instrumentation as discussed in the original proposal. The type of reader needed is more complicated than the visual readers studied during this phase I part of the project and we are currently exploring the possibility of prototyping such an instrument with the appropriate engineering experts.

NB: Diagrams of format are identical to gold assay, with substitution of fluorescent conjugate for gold. Methodology for making the test is also identical to gold assay. Therefore, we will not repeat the information or the diagrams.

2. Hand-Held Reader System

As noted in our prior reports, we have been working with a reader obtained from Source Instruments. This reader was used in the following manner:

The strip was placed in the tray at a preset position (for reproducibility). This meter scans a 1 cm portion of the strip. The data recording procedure provided was loaded onto a PC. The data recorded are position versus intensity measurements. These, as well as any other parameters for data intake, are not adjustable with this specific instrument. For example, the zero is predetermined internally, and we cannot offset background colors or increase sensitivity. This is the major drawback with this prototype since we cannot alter data input at all. The strip is then scanned automatically when the start button is hit. The data then is stored in the PC for later manipulation. We are thus provided with the computer graph intensity versus length and must then integrate the area under the curve for the test line. We need to use our own program for this as the manufacturer does not have integration software available at this time. After collecting data at several antigen levels and doing the data integration, we then plot the integrated signal versus antigen concentration. The plot shows sigmoidal type results at the low end of the curve. This is probably due to the signals' relative intensity being measured. **(See Attachment A for sample graphing)** We probably should take the log function and plot that so as to be measuring absorbency. However, since we cannot zero the instrument, we decided not to attempt that procedure at this time.

We have identified and obtained an additional prototype color reader for evaluation. This reader comes from Biotronics Associates. It differs from the Source reader in several important aspects. It is adjustable for negative readings and it can be read both by absorption or transmission. The fact that you can re-zero the reader to different negative backgrounds makes it more reliable when the background of the strips are different such as when using serum samples with different background color or using strips with different gold conjugate levels (which may cause a higher or lower reddish color background across the entire strip). The wavelength, using this new reader, is also adjustable so that you are not looking at a wide range of colors. The earlier Source reader was not color adjustable and had an arbitrary zero level internally set which was not adjustable. Both the Biotronics and the Source readers are sensitive to the nanogram range using the hCG assay as a standard. However, the color intensity was linear with the Biotronics Associates reader in the entire range. The Source reader was observed to be nonlinear in the lower ranges. The Biotronics reader also has a direct readout and does not require a PC. The Biotronics reader does not scan but has a 5 mm orifice within which the line on the strip can be placed. The zero can be calibrated to a blank spot on a strip to correct for background color differences. We can get linear results measuring absorbency but find that great care must be taken in placing the strip correctly over the light orifice. A scanning method would seem preferable with the strips but using a more narrow orifice might alleviate some of the problem with the Biotronics. The line is approximately 1 mm in thickness, at this time, and we are using a 5 mm wide strip for these experiments.

We feel that we can combine the positive aspects of both readers and incorporate them into the next generation of instruments. Both instruments are approximately 10 inches square in shape 4 inches in height. The final prototype needs to approach hand-held size. Our experience with manufacturers that we have worked with makes us confident in the ability to progress further on an appropriate prototype.

3. Multi-analyte format

Our initial proposal included a plan to format the multi-analyte in a radial design. This, we felt, had potential for easy workability and readability. (For edification of designs, please see **Figures 2-7**). We optimized the radial assay format using both hCG and drugs of abuse assays. We used hCG and drugs of abuse because of their relevant ease of being obtained. The high volumes of reagents necessary for this procedure relegate the use of target reagents (SEB, ricin, plague, VEE, etc.) to only the final stages of development. We ran into significant problems with the radial assay which, at this point in time, have alerted us to the need to abandon it, if only temporarily, in favor of a strip format. It is certainly a possibility to go back to re-examine the radial design as part of a Phase II program. The drawbacks that we ran into with the radial format were:

- we required approximately 25 fold more conjugate per assay using this radial format since most of the conjugate does not migrate specifically to the target assay spot.

- The sensitivity of the assays was diminished when we used a common anti-mouse IgG conjugate with free individual monoclonal antibodies for each specificity instead of a different conjugate for every assay. Therefore we needed to use an excess of conjugate for every assay using conjugate of each specificity.

- We found that we used approximately 30 times the amount of the nitrocellulose membrane which is a relatively expensive item. The circular/radial nature of this format requires us to die cut out circles from sheets (rather than the narrow strips normally used where rolled stock can be used directly). This wastes even more membrane and consumes production time.

- The cost per test for the membrane alone is approximately 90 cents per test, versus 40 cents in the strip format.

- We also found that the reagent dispensing and processing equipment we currently have is not sophisticated enough to use directly in the radial design and that much more sophisticated production equipment would be necessary. The approximate cost for such equipment is estimated to be $750,000 and would be required to be custom made.

Figure 2 ANTIGEN DETECTION RADIAL ASSAY

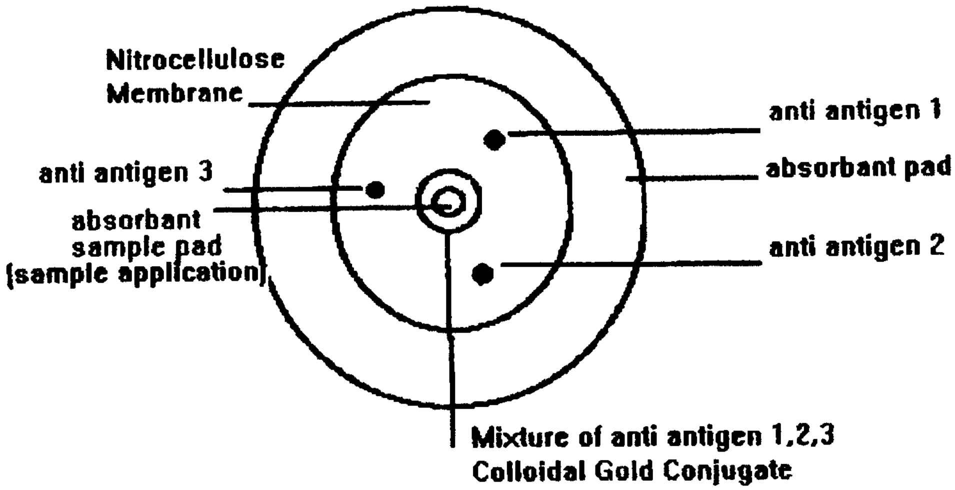


Figure 3 ANTIBODY DETECTION RADIAL ASSAY

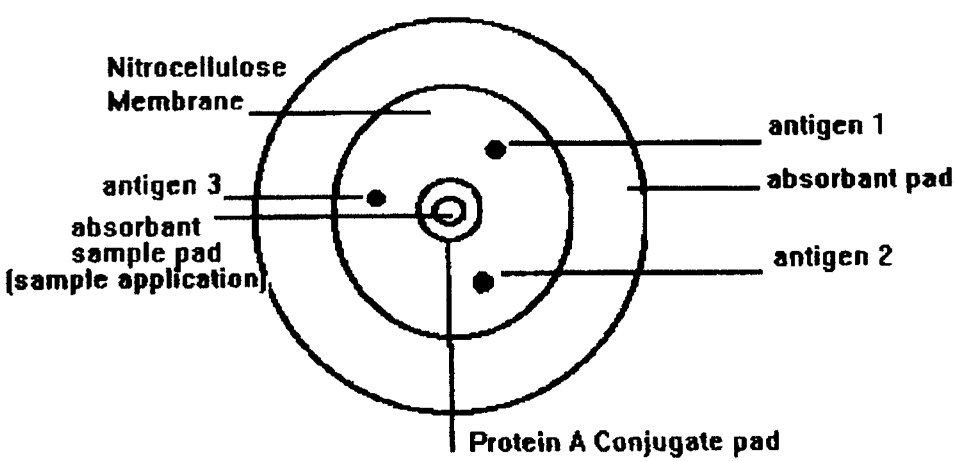


Figure 4

ANTIBODY ANTIGEN ANTIBODY GOLD COMPLEXATION

EXAMPLE 1

antigen 1 and 2 positive
antigen 3 negative

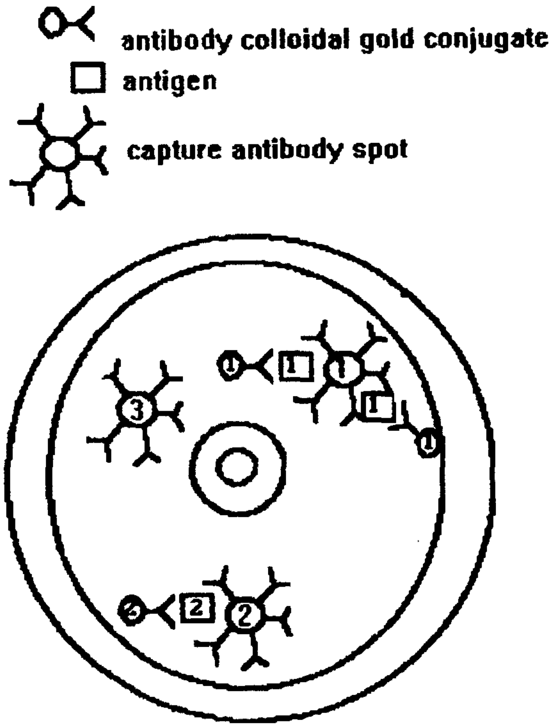


Figure 5

SAMPLE RECONSTITUTED CONJUGATE

EXAMPLE 1

antigen 1 and 2 positive
antigen 3 negative

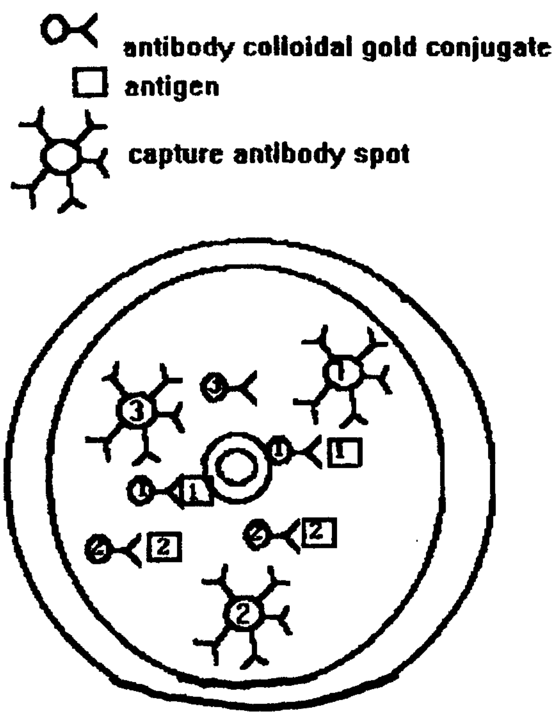


Figure 6

ANTIGEN ANTIBODY PROTEIN A COMPLEXATION
EXAMPLE 2

antibody 1 and 2 positive
antibody 3 negative

antibody colloidal gold conjugate

protein A conjugate

antigen capture spot

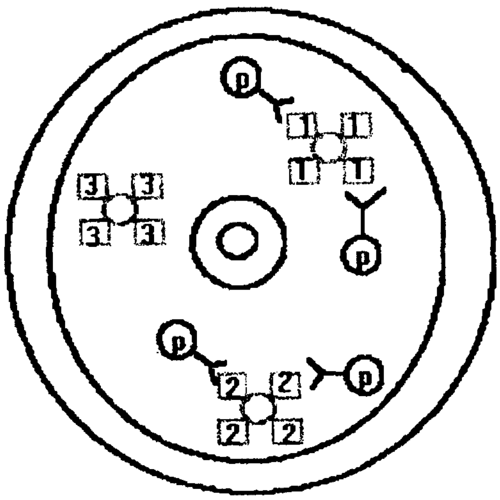


Figure 7

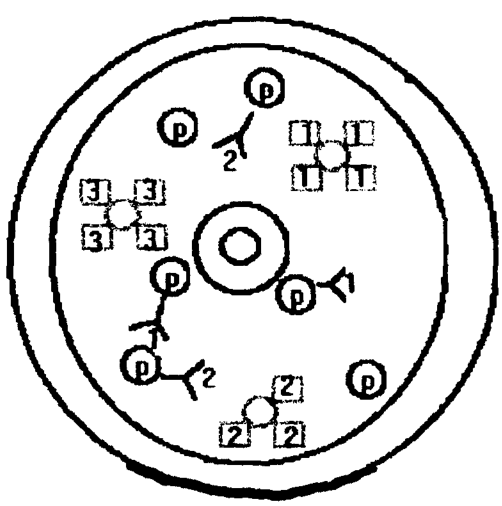
SAMPLE RECONSTITUTED CONJUGATE
EXAMPLE 2

antibody 1 and 2 positive
antibody 3 negative

antibody colloidal gold conjugate

protein A conjugate

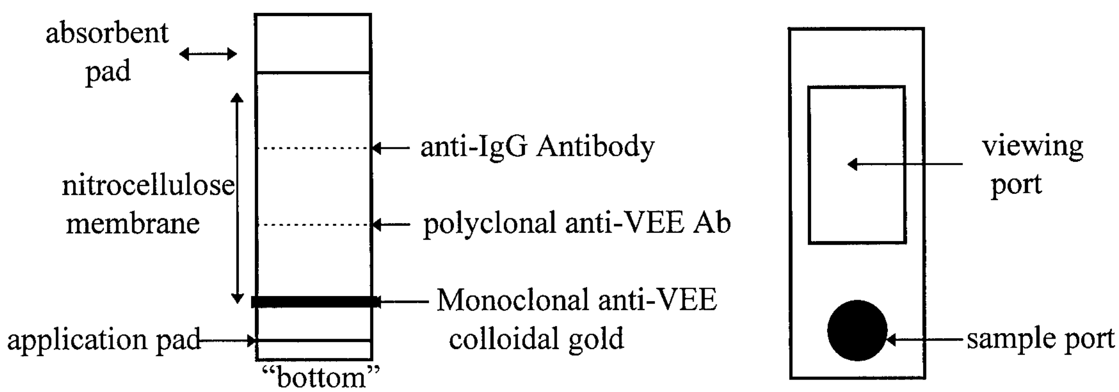
antigen capture spot



Multi-analyte format (Con't)

For all of these reasons we decided to explore the multi-analyte strip format for the remainder of the project. This format may be more useful for antibody assays than antigen assays, in the future, since only a single conjugate would be used for all the specificities on the device. As a first step in this multi-analyte format design, we optimized, in individual strip format, tests for ricin, SEB, plague, and VEE. **(Figure 8)**

(Figure 8) **VEE Assay ****



****Exact same format for ricin, plague, and SEB**

The sensitivities of these current generation of the individual assays were acceptable, although we would like to work to increase sensitivity.

The next step in the multi-analyte strip development was to spray all four assays on an individual strip and optimize the mixture. **(Figure 9)** We have observed some nonspecific binding of one colloidal gold conjugate with another capture antibody and need to optimize conditions where these antibodies and conjugates do not have any interactions. We see this problem frequently with assays made from mixtures of antibody from different source animals where there is heterophile type interactions in which one species has antibodies or specific interactions with another species. Normally each assay is optimized individually and the buffer in the absorbent pad and blocking of membranes for each assay are optimized in separate procedures to prevent these type of interactions.

Other, more tedious methods, for reducing this type of nonspecific interaction are:

- to individually absorb out these heterophile interactions using agarose antibody columns to remove these antibodies from the already affinity purified polyclonal antibodies.

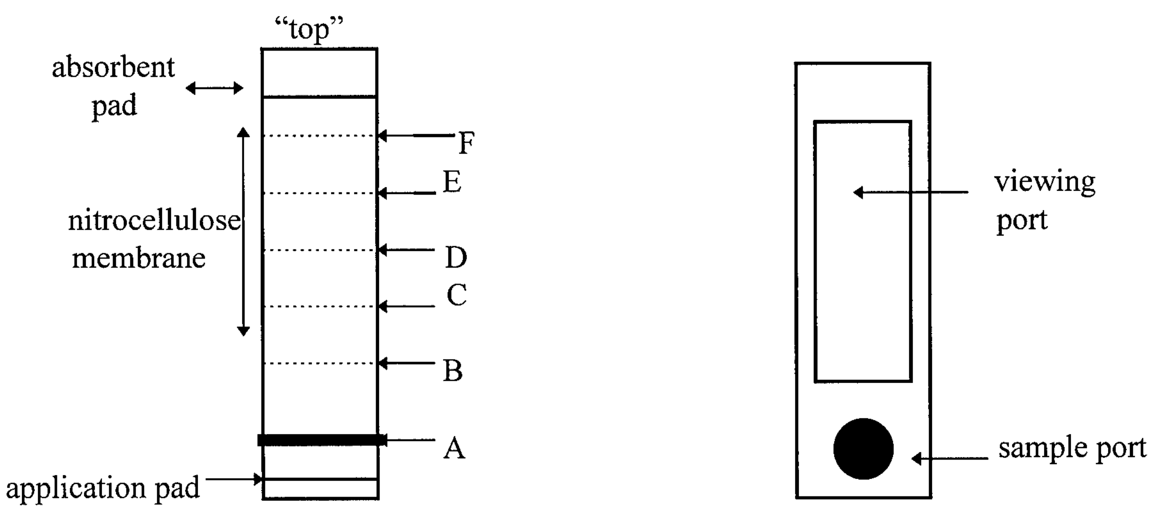
- to cleave either the monoclonal or the polyclonal antibodies and form Fab type fragments since removing the Fc region of the antibodies tends to remove these interactions also.

We are in the process of first optimizing the buffers before we use either of these two modifications of the antibody treatments since the amount of reagent available is relatively small. Because of scaling-up problems associated with these procedures, both these protein purification and modification methods tend to be reagent consuming and are only feasible with quantities over 100 mg of purified reagent.

An example of a multi-analyte format for antibody detection (using hanta and dengue antigens) is provided in **Figure 10**.

(Figure 9)

Multi-Analyte Assay for Antigen Detection

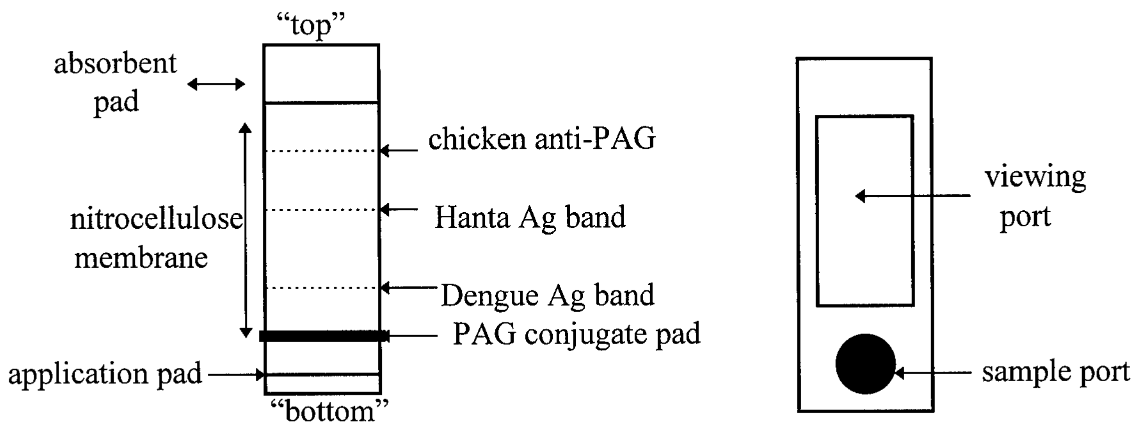


KEY: A = mixture of monoclonal anti - VEE, ricin, SEB, plague colloidal gold conjugates

B = polyclonal anti-plague band
C = polyclonal anti-SEB band
D = polyclonal anti-ricin band
E = polyclonal anti-VEE band
F = anti IgG antibodies band (control)

(Figure 10)

Multi-Analyte Assay for Antibody Detection



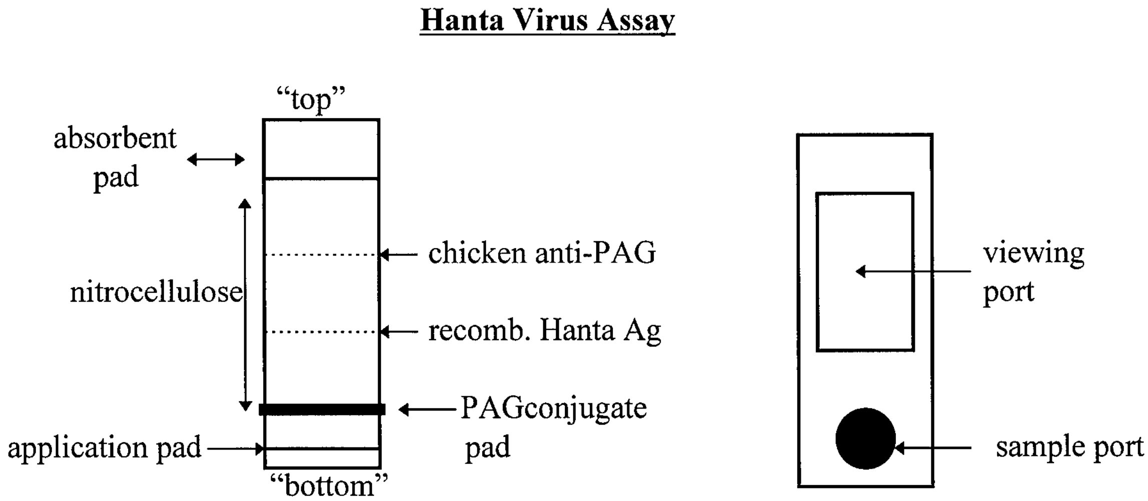
4. Hanta virus and dengue virus assays

4a. Hanta virus assay (Figure 11)

In our efforts to develop a prototype for this test, we have evaluated two sets of hanta antigens furnished by the Army and have found, in our system, that they are immunologically inactive, testing against currently available clinical samples of positive sera obtained from USAMRIID. We have investigated the possibility that there are chemicals in the system which would make them incompatible with our nitrocellulose binding and have concluded that this is not the problem. These antigens must lose their immunological activity upon binding to the membrane, be contaminated with an inactive protein or other form of the antigen, or have very low immunological activity relative to antigens used successfully in this type of assay format. We are currently waiting for additional material from the Army.

We have found, however, that a recombinant antigen, prepared in E.coli, from a private South Korean company (Che Dang, Seoul, S. Korea) seems to work very well. When using this recombinant antigen we can produce a test which gives us excellent specificity and sensitivity. By coating approximately 500 ng of this antigen per test strip and using a protein A colloidal gold we have been able to consistently detect antibody positive test samples. Clinical samples available from domestic sources are relatively rare although we have observed positive results with the two samples made available to us by USAMRIID. The only information we have on those 2 samples is that they should be strongly positive but are quite old. Ten problematic type negative samples used in-house have shown negative results (i.e., no false positives). Since they have access to many more samples at the Korean NIH then we do here in the States, we have sent 90 tests to Korea for their evaluation. They have found that out of the 70 positive samples tested thus far they observed 68 as clear positives and had not observed any false positives.

(Figure 11)



In the interim, we have made several improvements to increase sensitivity and have provided Korea with approximately 120 more assays for their continued evaluation. We have also supplied the Army with samples of both the raw Korean antigen and tests for evaluation at USAMRIID. The antigen supplied to us by the S. Koreans is estimated to be only about 50% pure. They are preparing a more pure antigen for us, to see if there is any further improvement that can be achieved in the assay.

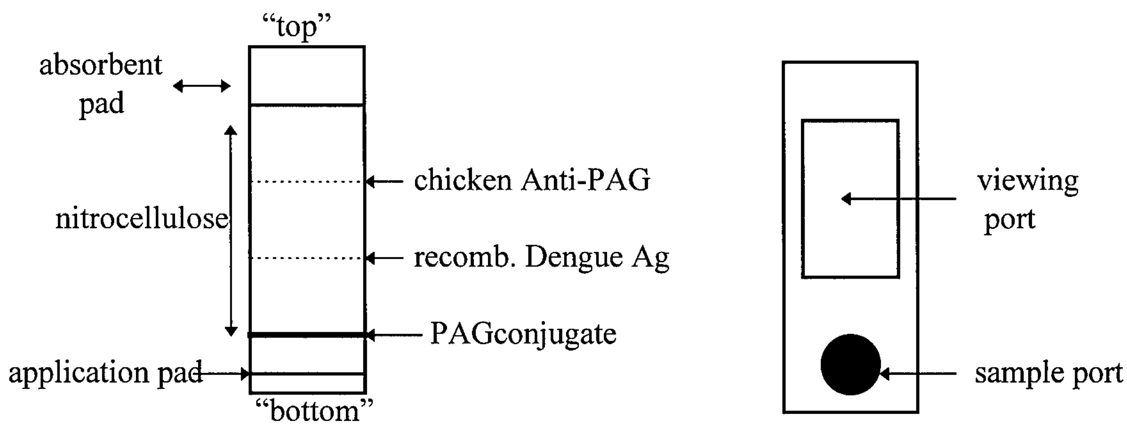
Specific methodology for hanta assay - The hanta test was formatted in the following manner. The antigen was coated at 1 mg/ml at 1 microliter per cm on SR Millipore nitrocellulose membrane in PBS The card was blocked with BSA Sucrose PBS and air dried. The colloidal gold used was a Protein A conjugate with a 40 nm particle size at an OD of 50 at 2 ul/cm. This was the maximum concentration of antigen available at this time. The assay buffer was applied to the absorbent sample pad. The buffer found optimally to work was pH 9.3 200 mM Sodium Borate, 0.% BSA, 1% Triton. The assay was found to require the addition of E coli extract (provided from the Korean antigen source) at 10% in passive gold diluent. This mixture was found to be necessary in at least a 25% ratio to the total volume of the sample in order to remove all false positives from problem negative samples. Evidently enough E. coli debris is left in this antigen preparation to give false positives with individuals with a titer to E coli.

4b. Dengue Assay (Figure 12)

As part of our attempt to develop a working assay for dengue antibody detection, we have evaluated dengue antigen from four individual sources. Those were NMRI, WRAIR, Rutgers University, and a Hawaiian biotechnology company (Hawaii Biotechnology Group, Inc.).

(Figure 12)

Dengue Virus test



The antigen sources varied in the following manner:

1. The Navy antigen (NMRI) was a native preparation partially purified by centrifugation. Most antigens processed in this manner do not work well in our type of format unless the typical antibody titers are very high. This preparation was not purified and contained media and animal sera straight from production. In this type of membrane format we normally require very pure specific proteins since we are observing direct binding and cannot use enzyme or other systems where the signal can be amplified. We saw very low reactivity with this preparation.

2. The Army (WRAIR) antigens are recombinant antigens for dengue 1 and 2. We do not know what part of the target has been produced (although we assume it is the entire envelope protein) but we do not see any reactivity with the clinical samples made available to us by WRAIR.

3. The antigens obtained from Rutgers University were small synthetic peptides of approximately 8 amino acid units long conjugated to BSA. I believe that

these were too short to be practical especially since you normally need about 15 amino acid residues to determine an epitope site and the chances of finding each antigenic site which patients may have titer to are slim. We have not seen any reactivity with these preparations in trials in our lab.

4. The final reagents are from a vaccine company in Aiea,Hawaii, Hawaii Biotechnology Groups, Inc., and are again recombinant viral antigens prepared in baculovirus. We did our initial testing with antigen from dengue-2, although we now have antigens for dengue 1, 3, & 4 which we have not as yet formatted on our assays. Dengue 1 and 2 are similar in nature so I am not surprised that we have already seen reactions on positive dengue 1 & 2 sera with the dengue 2 antigen. This antigen did produce good signals with all positive samples available to us from both WRAIR and the CDC. **(Information on those sera samples is provided as Attachment D)**. Using a cruder preparation of the Hawaiian antigen did not work as well for sensitivity and we lost some of the positive samples. I believe we will need to stick with the purity level they initially supplied us with. Currently this assay picks up both IgG and IgM samples and we have shown 100% sensitivity and 100% specificity with 20 positive samples from the CDC and 2 serum pools. We have tested the assay using our panel of ten problematic negative samples and ten normal negative samples and have not observed any false positive results. We have sent samples to the CDC for further evaluation.

Specific methodology for the dengue assay - Using the antigen obtained from the Hawaii company, we applied at 1 mg/ml at 1 microliter per cm. The cards were blocked as the hanta assay. The same assay buffer was applied to the sample pads along with the same conjugate and amount of conjugate. We were not required to add any E coli extract or any other type of material such as detergents to the serum sample. However, in order to increase the assay time to five minutes using viscous samples, we found that it was necessary to dilute the sample 50:50 with passive gold diluent. If time for running and completing the test were not an important parameter, then neat serum was found to work just as well for specificity and sensitivity. The assay took up to 10 to 15 minutes for migration to occur with some samples when buffer was not added.

We may also, once the work is complete on all four dengue serotypes, format an assay with four separate bands representing those serotypes. Further development would aim to specifically identify separately the IgM and IgG reactions.

CONCLUSION

We have shown that we can achieve equivalent visual detection using fluorescent markers, although we had originally thought that we could exceed the visual detection already standard in our colloidal gold format. We have concluded that the problem here lies within the limits of human visual acuity and that to detect the greater levels of sensitivity that we still believe can be, or have been, achieved, that a specific UV reader is necessary. This would be fully investigated under a Phase II program. We have little doubt that we can eventually find the proposed 100-1000 fold increase in detection capability, bringing these simple assays to greater detection levels than current confirmatory testing capability.

Our reader system has met our expectations in this first go around. We have examined different readers and now feel confident that we can use this knowledge to modify existing, or engineer new, equipment that will result in a hand-held reader that can enhance interpretation of detection and provide quantification of results. In terms of ease of use, forward deployment, point of care, level of necessary expertise, this potential advancement in assay technology represents a clear advantage over the current body of knowledge and available equipment.

Temporarily discarding our efforts to approach a radial design for the multi-analyte format has enabled us to adjust our strip assay to the multi function design. We have not, as yet, worked enough with this assay to make any conclusive statements, but our previous experience with all of the component reagents bodes well for the eventual outcome.

Finally, the advancement of the hanta and dengue antibody detection assays has been a welcome product of our efforts. The early trials of both of these tests present us with a convincing potential for commercial marketability as well as DOD application.

Chart7

ATTACHMENT A

3rd standard curve

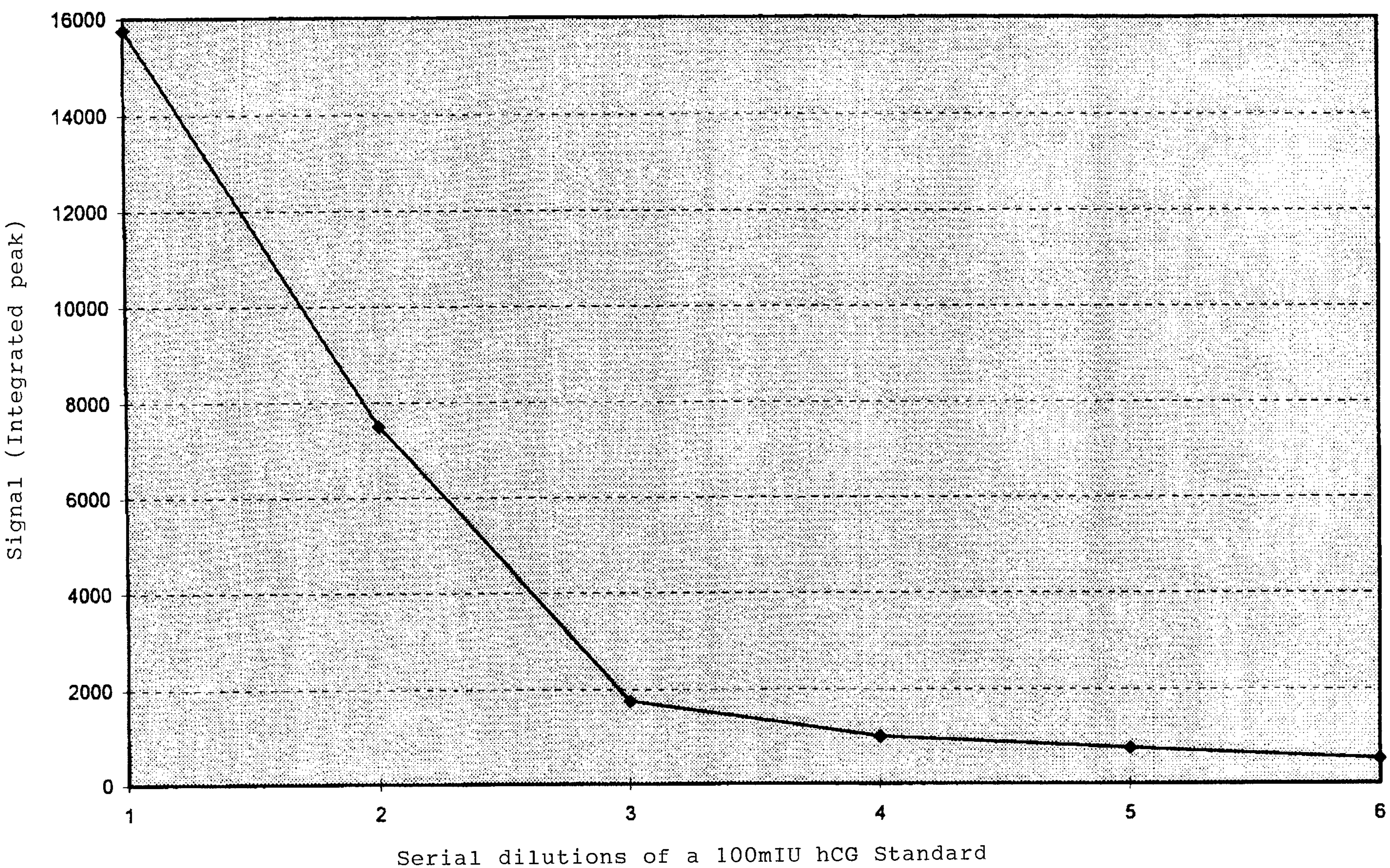
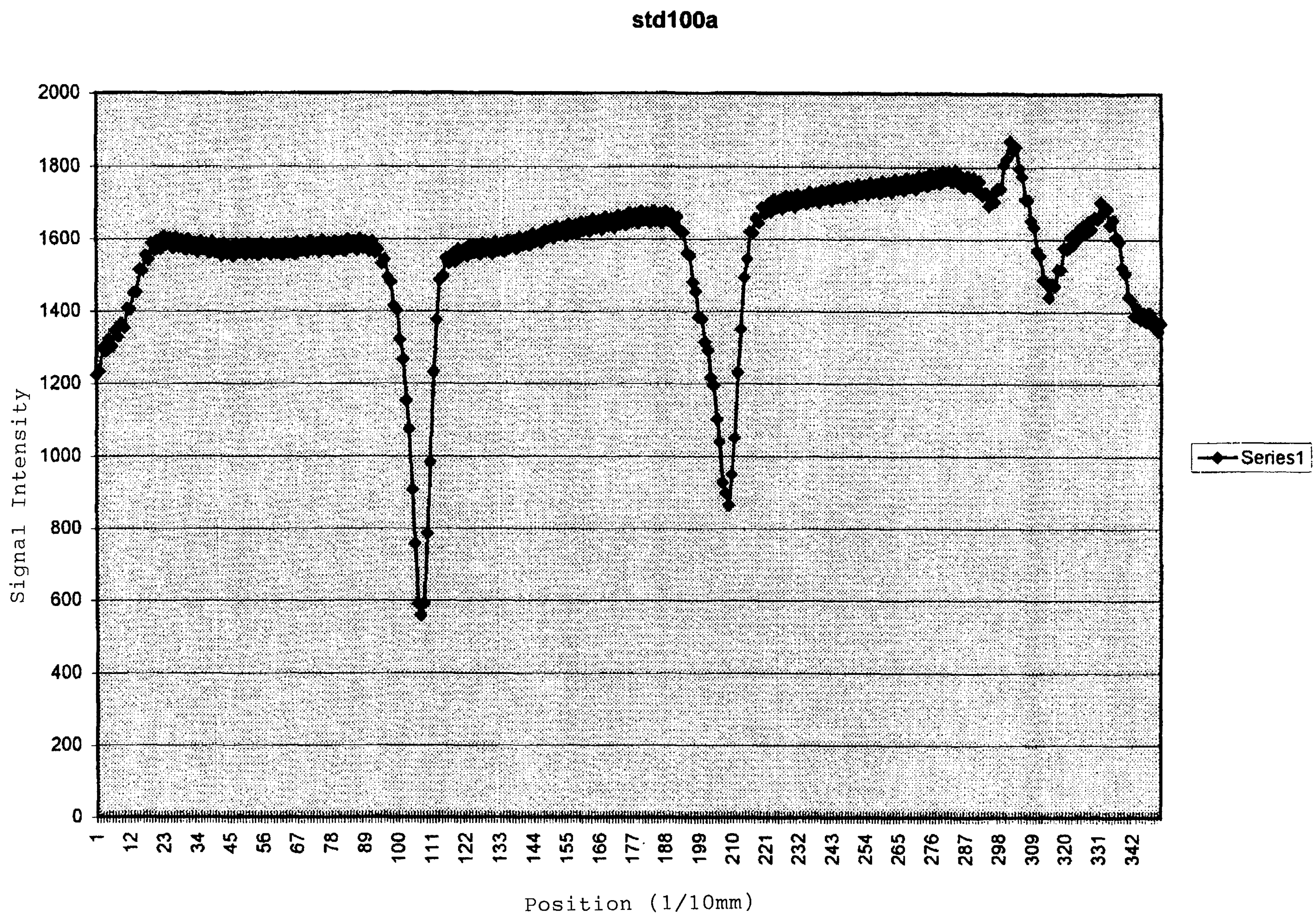
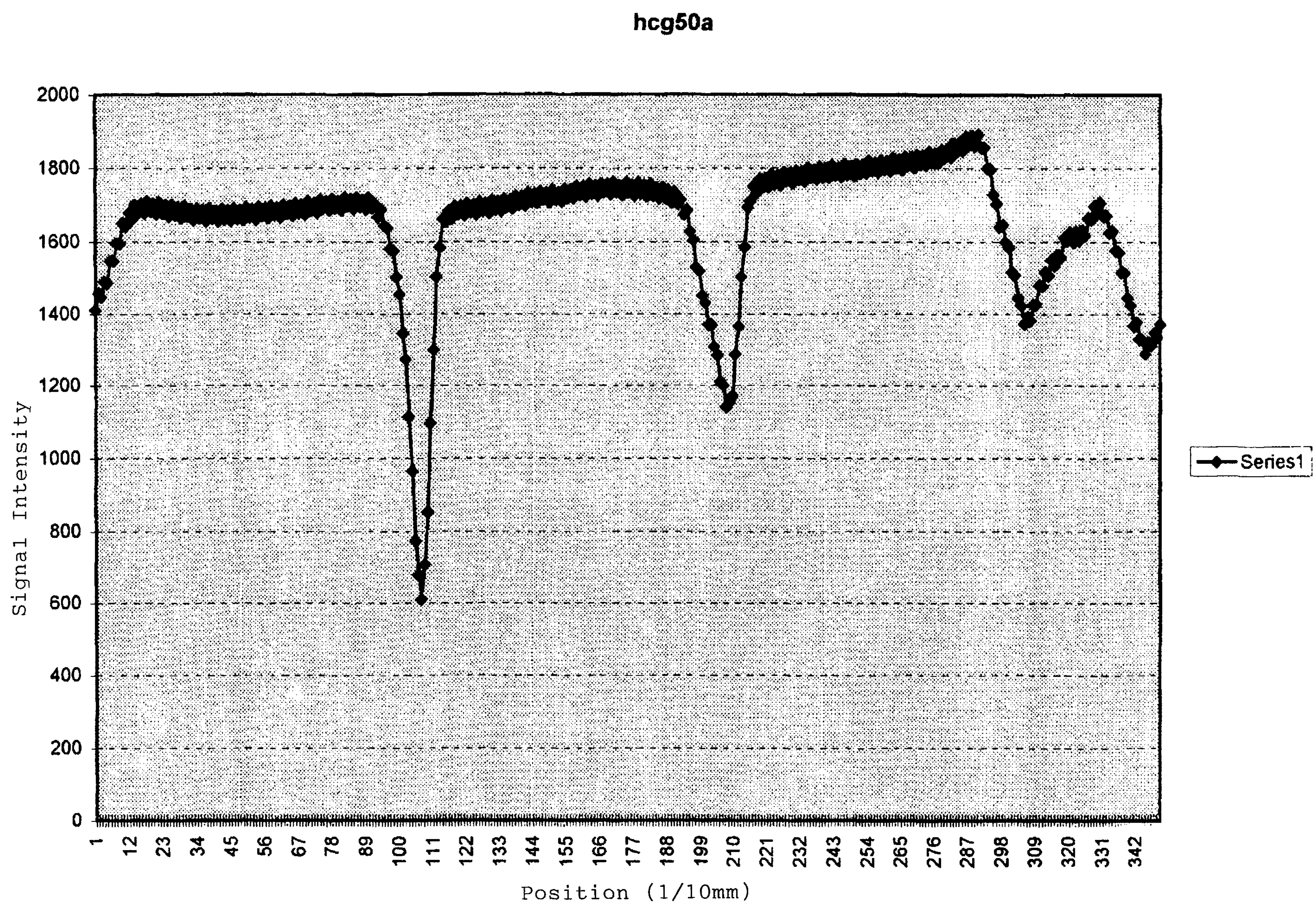


Chart1



std100a
Signal Intensity
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1
12
23
34
45
56
67
78
89
100
111
122
133
144
155
166
177
188
199
210
221
232
243
254
265
276
287
298
309
320
331
342
Position (1/10mm)
Series1

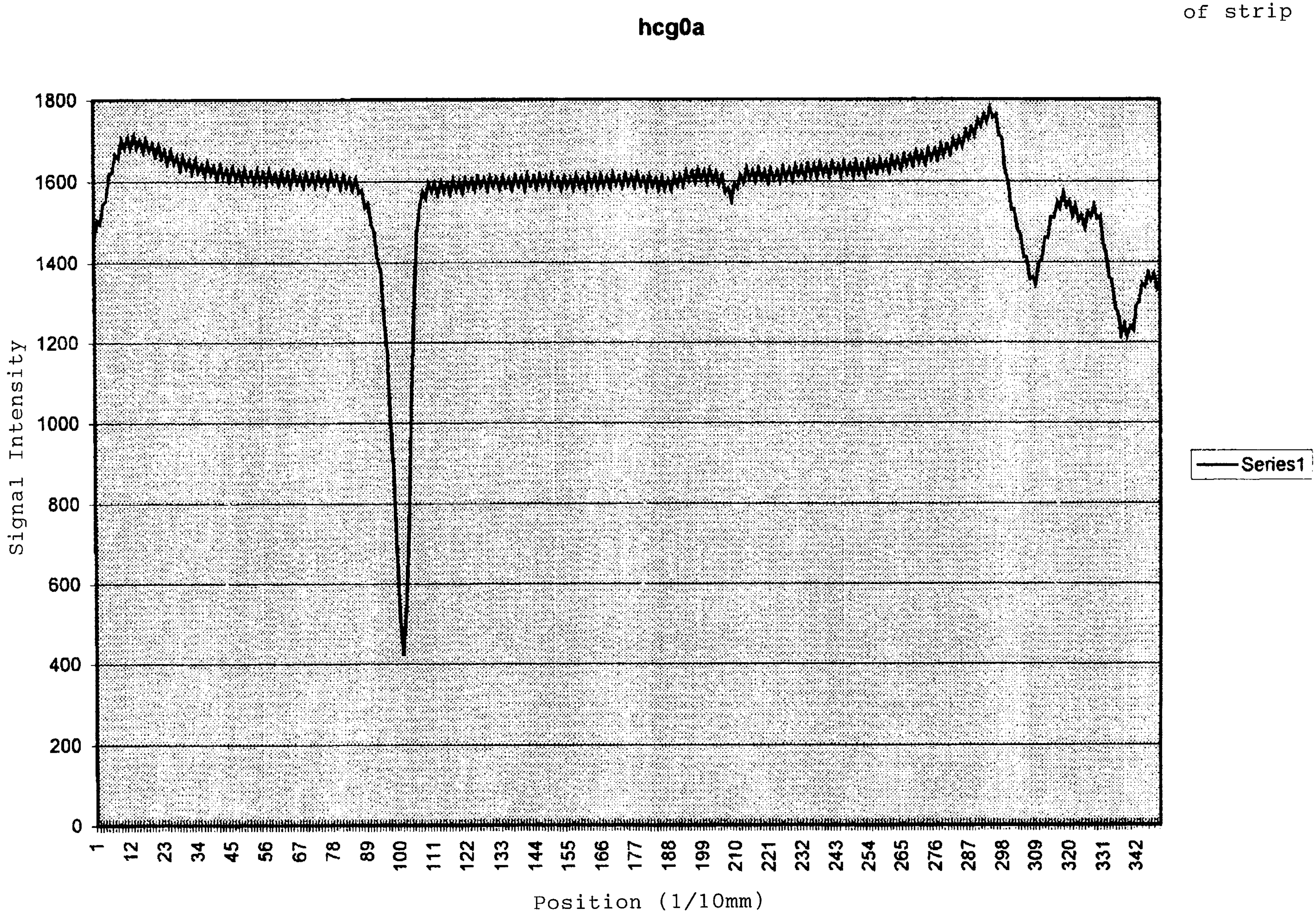
Chart1



hcg50a
Signal Intensity
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1
12
23
34
45
56
67
78
89
100
111
122
133
144
155
166
177
188
199
210
221
232
243
254
265
276
287
298
309
320
331
342
Position (1/10mm)
Series1

Chart1

Actual scan
of strip



Arista Biologicals, Inc.
115 Research Drive Bethlehem, PA 18015 phone(610)861-6901 fax(610)861-8247

ATTACHMENT B

March 17, 1997

Cheryl Lowery
Contract Specialist
R&D Contracts Branch B (CAL/dlh)
USAMRAA
Fort Detrick, MD 21702

Subject: SBIR Contract No. DAMD17-97-C-7027

Dear Ms Lowery:

We are interested in amending the above referenced SBIR contract to allow our company to receive and work with some additional unique military-relvant agents and reagents. WE can accomplish this revision to the original contract provisions within the current negotiated amount of $98,150.00 and the Phase I completion date of May 24, 1997. The requested revisions are as follows:

a. Delete the effort for enterotoxigenic *Escherichia coli* detection in stool specimens because the required reagents are not currently available.

b. Add VEE virus detection. IN addition to usefulness for multi-analyte detection, this would be a good model for fluorescent labeling of the detector antibody.

c. Add VEE virus IgM tests as a candidate for multi-test for IgM antibodies to dengue viruses and hantaviruses.

d. Add plague F1 antigen detection as a candidate for multi-test for BW agents in serum.

e. Add anthrax-protective antigen (PA) detection as a candidate for multi-test for BW agents in serum.

f. Add ricin detection as a candidate for multi-test for BW agents in serum.

g. Add Staphylococcal enterotoxin B (SEB) detection as a candidate for multi-test for BW agents in serum.

Thank you for your consideration in this matter. If you need more information, please contact me at 610-861-6901 or contact Dr. Greenspan at 908-730-8717 or fax to him at 908-735-6842.

Respectfully,

Dr. John Colanduoni
President
Arista Biologicals, Inc.

cc: Lt. Col. Roger Parker, USAMRIID

Majesco DENGUEVIRUS
A One Step Denguevirus Antibody Detection Test

A rapid, one step test of the qualitative detection of IgM and IgG antibodies to Denguevirus in serum or plasma.

For professional in vitro diagnostic use only.

INTENDED USE

The Majesco One Step Denguevirus Test is a rapid chromatographic immunoassay for the qualitative detection of antibodies to Denguevirus in serum or plasma.

SUMMARY

Denguevirus is a member of the flavivirus family , endemic in tropical and subtropical areas, and a major cause of morbidity and mortality in those regions. There are 4 identified sero-types of the virus (D1, D2, D3, D4), although the vast majority of infections are caused by just 2 (D1,D2), with many individuals exhibiting primary infection by either of the 2 major types and secondary infections by the other. The clinical spectrum of the disease ranges form asymptomatic to severe hemorrhagic/ shock syndrome. Key to lowering morbidity and mortality is aggressive treatment in the early stages, with diagnosis dependent upon early identification of antibodies to the Denguevirus.
The Majesco Denguevirus One Step Test is a rapid test utilizing proprietary recombinant antigens to qualitatively detect the presence of both IgM and IgG antibodies to the D1 and D2 sero-types of the Denguevirus.

PRINCIPLE

The Majesco Denguevirus One Step Test is a qualitative, solid phase, immunoassay for the detection of antibodies specific to Denguevirus 1 & 2 in serum or plasma. The membrane is pre-coated with Denguevirus recombinant antigens on the test line region and anti-conjugate on the control line region. During the testing, the serum or plasma sample reacts with the dye conjugate (anti-human antibody-colloidal gold conjugate) which has been pre-coated within the test device. The mixture then migrates upward on the membrane chromatographically by capillary action to react with the recombinant antigen on the membrane and generate a red line. Presence of this red line indicates a positive result, while its absence indicates a negative result. Regardless of the presence of detectable antibodies, as the mixture continues to migrate across the membrane to the immobilized anti-conjugate area, a red line at the control line region will always appear. The presence of this red line serves as verification for sufficient sample volume and proper flow and as a control for the reagents.

STORAGE AND STABILITY

Store as packaged in the sealed pouch at room temperature (20-30^{0}C). The kit is stable within the expiration date printed on the label. DO NOT FREEZE or use beyond the expiration date.

MATERIALS PROVIDED

- 40 Majesco Denguevirus one step test devices
- 40 Disposable sample droppers
- 1 set of instructions for use

MATERIALS REQUIRED BUT NOT PROVIDED

- Vacutainer tubes for serum or plasma collection
- Centrifuge

ATTACHMENT C

- For professional in vitro diagnostic use only. Do not use after the expiration date
- Do not eat, drink, smoke in the area where the specimens and kits are handled
- Handle all specimens as though they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when assaying samples.

SPECIMEN COLLECTION

- The Majesco Denguevirus One Step Test can be performed on serum or plasma only
- Remove the serum or plasma from the clot of red cells as soon as possible to avoid hemolysis. Only clear, non-hemolyzed specimens should be used.
- Testing should be performed as soon as possible after sample collection. Do Not leave samples at room temperature for prolonged periods. Specimens can be refrigerated at 2-8ºC for up to 3 days. Otherwise specimens should be stored at below -20ºC.
- Bring specimens to room temperature prior to testing.The frozen specimens must be completely thawed prior to testing. Specimens should not be repeatedly frozen and thawed.
- If specimens are to be shipped, they should be packed in compliance with Federal regulations covering the transportation of biologic agents.

TEST PROCEDURE

Read the entire procedure carefully prior to performing any tests. **Allow test devices and serum samples to equilibrate to room temperature (20-30ºC) prior to testing.**

1) Remove the Majesco Denguevirus test device from foil pouch (bring the test to room temperature before opening the pouch). Use device as soon as possible but within 1 hour after removal from pouch especially if the room temperature is more than 30ºC and in high humidity environment.

2) Place the test device on a clean and level surface. Holding the dropper vertically, dispense three full drops of serum (~150ul) without air bubbles into the sample well "S" of the test device.

3) Wait for red lines to appear. The test should be read in approximately 10 minutes. It is significant that the background is clear before reading the test, especially when samples have low antibody titers, and only a weak line appears in the test region (T). Do not interpret results after 30 minutes.

QUALITY CONTROL

A procedural control is included in the test. A red line appearing in the control region (C) is considered as internal positive procedural control. A clear background in the result window is considered as internal negative procedural control.
It is recommended that appropriate positive and negative serum or plasma sample controls be included in each days testing to verify proper test performance.

INTERPRETATION OF RESULTS

POSITIVE: Two distinct lines will appear, one in the test region (T) and another in the control region (C).

NEGATIVE: Only a single red line appears in the control region (C). No apparent red or pink line appears in the test region (T).

INVALID: Control line fails to appear which means improper testing procedure or deterioration of reagents probably has occurred. In any event, repeat the test. If the problem persists, discontinue use of lot. Immediately and contact your local distributor.

NOTES: The shade of red color in the test line region (T) will vary depending on the concentration of antibodies present. However, neither the quantitative value nor the rate of increase in antibodies can be determined by this qualitative test.

LIMITATION OF THE PROCEDURE

Majesco Denguevirus test is for in vitro diagnostic use only. This test should be used for the detection of antibodies to Denguevirus sero-types 1&2 in serum or plasma samples.

The test will only indicate the presence of antibodies to the specified Denguevirus strains in the specimen and should not be used as the sole criteria for the diagnosis of denguevirus infection. As with all diagnostic tests, a result must be considered in conjunction with other clinical information available to the physician. The Majesco Denguevirus test cannot detect extremely low concentrations of antibodies in specimens. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is required. A negative result at any time does not preclude the possibility of Denguevirus infection.

EXPECTED VALUES

Majesco Denguevirus test was compared to leading commercial EIA tests for denguevirus and the correlation between the systems was 98%.

PERFORMANCE CHARACTERISTICS

Sensitivity
Majesco Denguevirus test was tested with a sensitivity panel containing sera positive to the D1 and D2 sero-types of the virus with resultant appropriate sensitivity to both strains.

Specificity
Recombinant antigens used for the Majesco Denguevirus test were developed in baculovirus. The two primary sero-types (D1, D2) produced positive results on the Majesco test. Specificity was tested using multiple well characterized samples of D1 & D2 with no incidence of false positive results.

Precision
Intra-Assay
Within run precision was determined by using 15 replicates of three specimens (2 containing antibodies to D1&D2 and 1 without antibodies to either). The positive and negative values were correctly identified 98% of the time.

Inter-Assay
Between run precision was determined by using the same three specimens in 15 independent assays and with three different lots of Majesco Denguevirus test reaction cards over a 3 month period. The negative and positive values were correct 98% of the time.

		Reference Denguevirus Method	
		positive	negative
Majesco	positive	145	5
Method	negative	0	150

BIBLIOGRAPHY

1. Baron, EJ, Chang, RS, et al. Medical Microbiology. J. Wiley. 1994.

Manufactured for:
Majesco Biologicals, Inc.
115 Research Drive
Bethlehem, PA 18015
Tel: (610)861-6901
Fax: (610)861-8247

ATTACHMENT D

Sera Samples used to evaluate Dengue Assay

NB: All samples reacted with the assays suing the Hawaii Bio. antigen.

From the DOD (WRAIR):

Sample	Titre IgM	Titre IgG
1041	50	600
1046	12800	1600
1068	12800	6400
1021	50	6400
1136	12800	6400
1146	50	400
1080	50	12800
1029	1600	1600
1138	12800	1600
1137	12800	12800
1122	50	1600
1037	50	12800
1123	100	400
1047	12800	1600

From the CDC:

NB: The samples from the CDC were sent in 2 shipments, 2 months apart, and were run in separate in-lab trials with different batches of tests and different batches of antigen reagent. Although there are some repeats in sample ID numbers, this amounts to 2 separate studies and the results, as given above, show consistency over time, from batch to batch of tests, and from batch to batch of reagent.

The heading designations in the first sampling group are defined as:

DPO = days post onset of symptoms
O.D. = optical density in the standard MAC-ELISA

First grouping of samples:

Lab #	DPO	O. D.	Type of Sample
334123	14	1.19	Primary Dengue 1
333870	5	0.72	Secondary Dengue 1
335445	10	0.26	"
335533	7	0.94	"
335704	17	0.93	"
294987	21	0.43	Primary Dengue 2
332823	8	0.35	Secondary Dengue 2
332960	8	0.53	"
332980	12	0.71	"
333797	11	1.83	"
335581	13	1.36	"
335927	16	0.56	"
332977	6	0.30	Undefined sera, D-1,2,4
332978	6	0.61	"
332983	5	0.65	"
333157	5	0.40	"
333163	6	0.39	"
333166	6	0.45	"
333286	5	0.31	"
333320	8	0.93	"
333353	7	0.29	"
333354	4	0.70	"
333358	8	0.76	"
333359	4	2.28	"

Second grouping of samples (ID#s only provided by CDC):

primary dengue 1	secondary dengue 1
275558	335545
326250	335533
308224	335704
327327	
327483	

primary dengue 2	secondary dengue 2
334926	334022
336725	335581
294989	335927
	336102
	336230

ATTACHMENT E

PREPARATION OF IMMUNOCHROMATOGRAPHIC STRIPS

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4) SOP 04 003..........PREPARATION OF SOLUTION "B" AND TREATMENT OF POLYESTER MEMBRANE FOR SPRAYING WITH GOLD CONJUGATE

5) SOP 04 004..........PREPARATION OF ANTIBODY SOLUTION FOR SPRAYING

6) SOP 04 005......... LAMINATION OF GLUE AND MEMBRANE TO THE PLASTIC CARD

7) SOP 04 006......... APPLICATION OF ASSAY REAGENTS TO THE MEMBRANE

8) SOP 04 007......... SPRAYING GOLD CONJUGATE ONTO POLYESTER 2033

9) SOP 04 008......... ASSEMBLY OF THE DIAGNOSTIC TEST CARD

10) EXAMPLES OF BATCH RECORDS FOR MANUFACTURING

LIST OF SUPPLIERS

ABSORBANT PAPER

Schleicher and Schuell
10 Optical Ave.
P.O. Box2012
Keene N.H. 03431-2062
FAX 603-357-3627
TELEPHONE 800-245-4024
Contact ; Nancy Moran

CARDS (PLASTIC)

Estok Plastics co.
434 Whitehead Rd.
Trenton, N.J. 08619
FAX 609-584-1833
PHONE 609-584-1623
609-586-4336

G&L
Precision Die Cutting
1766 Junction Ave.
San Jose, CA 95112
FAX 408-451-1199
PHONE 800-327-4553
408-453-9400

CHEMICALS

Fisher Scientific
FAX 800-926-1166
PHONE 800-766-7000

Sigma Chemical Co.
St. Louis, MO.63178-9916
FAX 800-325-5052
PHONE 800-325-3010

DESSICANTS

Grace-Davison Chemical Co.
Curtis Bay, M.D.
PHONE 410-354-8954
Contact; Amy Lacher

DESSICANTS (continued)

United Dessicants
101 Christine Drive
Belen, N.M. 87002
FAX 505-864-9296
PHONE 505-864-6691

(we use ½ gm. packets)

Drierite
W.A. Hammond Drierite Co. Ltd.
Xenia, Ohio 45385
937-376-2927

(this is the granular type)

GLUE

Adhesives Research Inc.
P.O. Box 100
Glen Rock, PA 17327
FAX 717-235-8320
PHONE 717-235-7979
Contact; Karen Olsen

LAMINATOR

Bio.Dot. Inc. (see sprayers for address)

MEMBRANE

Millipore
80 Ashby Road
Bedford, MA 01730
FAX 617-275-5550
PHONE 800-225-3384
Contact; William Mc Kensie, Marketing Manager
Sheila, ext. 8662, Technical Service

POLYESTER 2033

Ahlstrom Filtration
Scientific Specialties Group
122 W. Butler St.
P.O. Box A
Mt. Holly Springs, PA 17065
FAX 717-486-4878
PHONE 800-326-1888
Contact;Jack Ramsey

POUCHES

Acon Biotech (Hang Zhou) Co., LTD.
148 Longjing Road
Hang Zhou, ZheJiang
P.R. of China 310013
contact; Feng Lin
(suppliers of large foil pouches)

POUCHES (continued)

Process Medical Systems
25 East Loop Rd.
Stonybrook, N.Y. 11790
FAX 516-444-8845
PHONE 516-444-8840

POUCHER

Bio.Dot., Inc. (see sprayers for address)

SLITTER/CUTTER

Bio. Dot., Inc. (see sprayers for address)

Kinematic Automation
Twain Harte, CA 95383
FAX 209-532-0248
PHONE 209-532-3200
contact; Ted Meigs

SPRAYERS

Bio. Dot., Inc. (Bio Jet sprayer)
17781 Sky Park Circle
Irvine, CA 92714
FAX 714 440- 3694
PHONE 714-440-3685
contact; John Geneau

Imagene Technology (Isoflow sprayer)
P.O. Box 667
Hanover, N.H. 03755
FAX 603-298-8513
PHONE 603 298-5632
contact; Tim Chow

ARISTA BIOLOGICALS

ADDITIONAL POUCH MANUFACTURERS

1. **ARVEY CORPORATION**
3500 North Kimball Avenue
Chicago, IL 60618
Tel No: (312) 463- 1400

2. **FASER INDUSTRIES**
Medical Packaging Group
Building P-1
Andrea Boulevard
Saddle Brook, NJ 07622
Tel No: (201) 797-2600

3. **KAPAK CORPORATION**
5305 Parkdale Drive
St. Louis Park, MN 55416
Tel No: (612) 541-0730

4. **KENPAK**
5416 Union Pacific Avenue
City of Commerce, CA 90022
Tel No: (312) 463- 1400

5. **LAMINATED FILM & PACKAGING**
5070 Industry Drive
Melbourne, FL 32940
Tel No: (305) 259-2131

6. **LPS INDUSTRIES**
113 North 13th Street
Newark, NJ 07107
Tel No: (201) 485- 4400

7. **MODERN PACKAGING**
Shreve Street
Mount Holly, NJ 08060
Tel No: (609) 267-5900

8. **RICHMOND TECHNOLOGY, INC**
1897 Colton Avenue
Rodlands, CA 92374
Tel No: (714) 794-2111

9. **RILEY & GEEHR, INC.**
2205 Lee Street
Evanston, IL 60202
Tel No: (708) 869-8100

10. **RJH & ASSOCIATES, INC.**
419 Eisenhower Lane South
Lombard, IL 60148
Contact: Rock A. Hauptman
Tel No: (708) 629-4010

11. **SPEC-FAB COMPANY**
1818 Rowland Street
Riverton, NJ 08077
Tel No: (609) 829-5100

12. **T.W KUTTER COMPANY**
91 Wales Avenue
Avon, MA 02322
Tel No: (617) 288-2600

ARISTA BIOLOGICALS, INC.

SOP 04 001 PREPARATION OF BLOCK BUFFER

VERSION NO: 1.0	SUPERSEDES: NEW FORMAT
PREPARED BY: B.SZILAGYI	DATE: 02/23/93
APPROVED BY: J.COLANDUONI	DATE: 02/23/93
REVIEWED BY: B.SZILAGYI	DATE: 09/04/95

1.0 PURPOSE

1.1 To describe the preparation of block solution.

2.0 SAFETY

2.1 Refer to the MSDS sheet appropriate to the material being handled. No safety precautions are required.

3.0 EQUIPMENT

3.1 Analytical balance.
3.2 Glass beaker of adequate volume to contain buffer.
3.3 Magnetic stir plate.
3.4 Magnetic stir bar.
3.5 Graduated cylinder.
3.6 pH meter.

4.0 MATERIALS:

4.1 Albumin, bovine, Sigma A-7906, or equivalent.
4.2 Sucrose, Sigma S-8501, or equiv.
4.3 Phosphate buffered saline (PBS) [.01 M Na2HPO4 dibasic, .15 M NaCl, pH 7.4]

5.0 PROCEDURE

5.1 Determine the volume of block solution needed. Note: 1000 ml. minimum for up to 50 strips membrane.
5.2 Pour entire volume of PBS into a glass beaker. Volume is same as final volume.
5.3 Weigh amount of albumin (BSA) to make 2.0% of final volume. Sprinkle BSA evenly over PBS and allow to dissolve without stirring.
5.4 Weigh amount of sucrose to equal 20% of final volume and stir until dissolved.
5.5 Adjust pH to 7.4 as necessary.
5.6 Store at 4-8 C refrigeration until use.

6.0 CLEAN UP

6.1 Wipe balance with damp cloth as necessary.
6.2 Clean used glassware by standard procedures.

ARISTA BIOLOGICALS, INC.

STANDARD OPERATING PROCEDURE

SOP 04 002 PREPARATION OF SOLUTION "C" FOR TREATMENT OF ABSORBANT PADS

VERSION NO: 2.0	SUPERSEDES: 6/24/93
PREPARED BY: B.SZILAGYI	DATE: 8/19/93
APPROVED BY: J.COLANDUONI	DATE: 8/19/93
REVIEWED BY: B.SZILAGYI	DATE: 9/07/95

1.0 PURPOSE

1.1 This procedure provides guidelines for the preparation of SOLUTION C and the treatment of absorbant paper.

2.0 SAFETY

2.1 Protective eyewear and clothing must be worn as indicated by the MSDS of the chemicals used.

3.0 EQUIPMENT

3.1 Analytical balance
3.2 pH meter
3.3 Magnetic stir plate and stir bar
3.4 Beaker
3.5 Tray

4.0 MATERIALS

4.1 Triton X-100, Sigma X-100 or equivalent
4.2 Sodium Tetraborate, Fisher S249-500, or equiv.
4.3 HCl 4N or NaOH to adjust pH
4.4 DI water
4.5 Absorbant paper 8"x10", Grade 903, Schleicher & Schuell
4.6 Gloves

5.0 PROCEDURE

5.1 Weigh the desired amount of 0.1 M SODIUM BORATE: 0.1 x 381.37 (formula wt) x ______liters final vol.= ____________grams sodium borate. Lot #______________.
5.2 Dissolve the above in final volume less 100 mls DI water.
5.3 Add the desired amount of TRITON X-100 10%: Final

volume in mls x 0.10 = vol. of Triton X-100 to be added.
_________mls. x 0.10 = _________ mls. Triton X-100.
Lot #_____________________________.

5.4 Dissolve throroughly with mixing.
5.5 Adjust the pH to 10.0.
5.6 Pour solution into a tray and briefly but thoroughly soak the absorbant paper sheet one at a time in the solution.
Wear gloves when handling the sheets.
5.7 Remove the sheet from the solution and blot the sheet between two dry absorbant sheets.
5.8 Airdry the sheets completely on racks, taking care not to deform the sheets.
5.9 Cut the sheets into strips 3/4" x 180 mm.

6.0 CLEAN UP

6.1 Store unused SOLUTION C in capped container at room temperature.
6.2 Rinse tray with DI water.
6.3 Store cut strips in a clean sealed container.

ARISTA BIOLOGICALS, INC.

STANDARD OPERATING PROCEDURE

SOP 04 003 PREPARATION OF SOLUTION "B" AND TREATMENT OF POLYESTER MEMBRANE FOR SPRAYING WITH GOLD CONJUGATE

VERSION NO: 2.0	SUPERSEDES: 06/24/93
PREPARED BY: B.SZILAGYI	DATE: 08/19/93
APPROVED BY: J.COLANDUONI	DATE: 08/19/93
REVIEWED BY: B.SZILAGYI	DATE: 09/07/95

1.0 PURPOSE

1.1 The purpose of this procedure is to provide guidelines for the preparation of SOLUTION B and the treatment of polyester membrane grade 2033 (Ahlstrom).

2.0 SAFETY

2.1 Protective eyewear and clothing is to be worn as suggested by the MSDS of the chemical indicated.

3.0 EQUIPMENT

3.1 Analytical balance
3.2 pH meter
3.3 Magnetic stir plate and stir bar
3.4 Beaker
3.5 Tray
3.6 Gloves

4.0 MATERIALS

4.1 Polyvinyl Alcohol (PVA) 30,000 - 70,000 MW, Sigma P8136
4.2 Sodium Phosphate, dibasic, Sigma S-0876, or equiv.
4.3 Triton X-100, Sigma X-100
4.4 Bovine Serum Albumin (BSA), Sigma A-7906, or equiv.
4.5 DI water
4.6 Polyester membrane, grade 2033, Ahlstrom Filtration

5.0 PROCEDURE

5.1 Weigh the desired amount of POLYVINYL ALCOHOL 0.5%.
Dissolve thoroughly with the application of

moderate heat with stirring. Lot #____________________.
Final volume in mls. x 0.005 = ______grams PVA.

5.2 Weigh the desired amount of SODIUM PHOSPHATE 50 mM.
142 x .001 x 50 x final vol. in liters = gm Na2HPO4
7.1 x __________L = __________gm Na2HPO4. Lot #_______________
Remove from heat and dissolve with mixing.

5.3 Add the desired amount of Triton X-100 1.0%.
Final volume in mls x 0.01 = mls Triton X-100.
________mls x 0.01 = _________mls Triton. Lot #______________
Mix thoroughly.

5.4 Cool solution to less than 40C.

5.5 Weigh the desired amount of BSA 5.0%.
Final volume in mls. x 0.05 = gms BSA.
_________mls x 0.05 = __________gms BSA. Lot #______________.

5.6 Add BSA to cooled solution and mix gently until dissolved.

5.7 Adjust pH to 7.4.

5.8 Pour Solution B into a tray and, wearing gloves, dip the polyester sheets into the solution to wet completely. Soak 1 hour at room temperature.

5.9 Shake off excess solution and blot the sheets between paper towels. Dry on racks.

5.10 Store treated sheets in a clean, covered container until ready to spray with the gold conjugate.

6.0 CLEAN UP

6.1 Unused Solution B may be stored under refrigeration at 4-8 C for up to three days.

ARISTA BIOLOGICALS, INC.

STANDARD OPERATING PROCEDURE

SOP 04 004 PREPARATION OF ANTIBODY SOLUTION FOR SPRAYING

VERSION NO: 1.0	SUPERSEDES: NEW FORMAT
PREPARED BY: B.SZILAGYI	DATE: 02/23/93
APPROVED BY: J.COLANDUONI	DATE: 02/23/93
REVIEWED BY: B.SZILAGYI	DATE: 09/09/95

1.0 PURPOSE

1.1 To prepare the antibody solution for spraying onto membrane.

2.0 SAFETY

2.1 Wear protective clothing, gloves, and goggles as appropriate to the nature of the antibody.

3.0 EQUIPMENT

3.1 Glass pipets and bulb
3.2 Graduated cylinder

4.0 MATERIALS

4.1 Phosphate Buffered Saline (PBS)
(0.01 M Na_2HPO_4, dibasic, + 0.15 M NaCl, pH 7.4)
4.2 Stock antibody

5.0 PROCEDURE

5.1 Calculate the final volume of antibody solution needed:
5.1.1 Volume of antibody solution in mls. = number of cards to be sprayed x microliters of antibody per card divided by 1000.
5.2 Dilute the stock antibody to desired concentration (AC) in PBS:
5.2.1 AC divided by the concentration of stock in mg/ml x final volume of antibody solution in mls. = vol. of stock antibody in mls. needed.
5.2.2 Volume of PBS in mls. = final volume in mls. - vol. of stock antibody in mls.

6.0 CLEAN UP

6.1 Wash cylinder in nonfilming laboratory soap, deproteinize between different antibody types with 0.1 NaOH,, and rinse thoroughly in DI water.

6.2 Place stock antibody, and the diluted working concentration of antibody solution under refrigeration at 4 - 8 C.

ARISTA BIOLOGICALS, INC.

STANDARD OPERATING PROCEDURE

SOP 04 005 LAMINATION OF GLUE AND MEMBRANE TO PLASTIC CARD

VERSION NO: 2.0	SUPERSEDES: 2/23/93
PREPARED BY: B.SZILAGYI	DATE: 09/09/95
APPROVED BY: J.COLANDUONI	DATE: 09/09/95
REVIEWED BY:	DATE:

1.0 PURPOSE

1.1 To describe the lamination of the glue and membrane components to the plastic card.

2.0 SAFETY

2.1 Observe proper safety precautions while using the cutter.

3.0 EQUIPMENT

3.1 Paper cutter or equivalent
3.2 Template

4.0 MATERIALS

4.1 White polystyrene cards, 2 1/4" x 180 mm, Estok Plastics.
4.2 Arcare 7841 1" double-coated 1 mil polyester film, liner both sides, Adhesives Research.
4.3 Arcare 7841 1/2" double-coated 1 mil polyester film, liner both sides, Adhesives Research
4.4 Arcare 7841 3/4" double-coated 1 mil polyester film, liner both sides, Adhesives Research
4.5 Membrane, High Flow Nitrocellulose, 1" x 180mm, unspecified pore size, unbacked, Millipore

5.0 PROCEDURE

5.1 Cut the Arcare rolls into strips 180 mm long.
5.2 Remove the protective liner from one side of the 3/4" strip.
5.3 Using the template, place the tape, glue side down, on the plastic card along the bottom edge of the card.
5.4 Remove the liner from one side of the 1" tape and place it on the card 3/4" from the bottom edge, taking care not to overlap with the 3/4" tape.

5.5 Remove the protective liner from one side of the 1/2" tape and place it glue side down along the top edge of the card just above the 1" tape.

5.6 Cut strips of membrane 1" x 180 mm long.

5.7 Wearing gloves to protect the membrane from oils, remove the top liner from the 1" tape and position the membrane onto the glue. Take care in positioning the membrane because once in place, it cannot be romoved without damaging the membrane. Make sure there are no bubbles in the membrane and avoid dust particles.
NOTE: Do not rub the surface of the membrane during positioning; instead cover the positioned membrane with a second plastic card and apply even pressure.

6.0 CLEAN UP

6.1 Place all unused tape into plastic bags for storage.

6.2 Store unused membrane in a protective container.

6.3 Store finished cards in a dust-free container until use.

ARISTA BIOLOGICALS, INC.

STANDARD OPERATING PROCEDURE

SOP 04 006 APPLICATION OF ASSAY REAGENTS TO MEMBRANE

VERSION NO: 1.0	SUPERSEDES: NEW FORMAT
PREPARED BY: B.SZILAGYI	DATE: 09/09/95
APPROVED BY: J.COLANDUONI	DATE: 09/11/95
REVIEWED BY:	DATE:

1.0 PURPOSE

1.1 To describe the application of antibody/antigen reagents to membrane, blocking and drying procedures.

2.0 SAFETY

2.1 Gloves should be worn at all times to protect both the technician and the membrane.
2.2 Protective clothing should be worn.

3.0 EQUIPMENT

3.1 Sprayer, Biodot, or equivalent
3.2 Incubator/dessicator 37C
3.3 Tray for blocking

4.0 MATERIALS

4.1 Prepared working concentration of antibody/antigen solution.
4.2 Cards, 2 1/4" x 180 mm, with laminated membrane
4.3 Paper towels
4.4 Blocking solution
4.5 Gloves
4.6 Compressed gas supply

5.0 PROCEDURE

5.1 Program the appropriate setting onto the sprayer.
5.2 Place the card on the platform and align the spray head over the desired line of spray. Secure the card with magnets.
5.3 Fill the resevoir with the antibody/antigen solution to be sprayed.
5.4 Turn on the gas supply and begin the spray, watching the spray pattern closely for consistency and accuracy.
5.5 Dry the sprayed cards on a rack in the incubator for 30 minutes at 37C.

5.6 Soak the cards face down in block solution for 30 minutes at 37C. Wear gloves when handling cards in block solution.

5.7 Remove the cards from the block solution and blot between paper towels membrane side down, taking care not to rub the membrane. Insufficient blotting may affect function of test strips.

5.8 Dry the blocked cards overnight at 37C then store in desiccated containers at room temperature. Optimal relative humidity in storage container should be 20% or less.

6.0 CLEAN UP

6.1 Thoroughly clean the spraying syringes and jets as recommended by the manufacturer.

6.2 Place all unused reagents into refrigeration at 4 - 8C.

6.3 Daily discard all unused block solution.

SOP 04 007 SPRAYING GOLD CONJUGATE ONTO POLYESTER 2033

VERSION NO: 1.0	SUPERSEDES: NEW FORMAT
PREPARED BY: B.SZILAGYI	DATE: 08/19/93
APPROVED BY: J.COLANDUONI	DATE: 08/19/93
REVIEWED BY: B.SZILAGYI	DATE: 09/09/95

1.0 PURPOSE

1.1 To describe the process for spraying gold conjugate.

2.0 SAFETY

2.1 Gloves and protective clothing should be worn.

3.0 EQUIPMENT

3.1 Sprayer, Biodot or equivalent
3.2 Incubator/dessicator set at 37C

4.0 MATERIALS

4.1 Prepared gold conjugate suspension
4.2 Polyester sheets, 2033 grade, treated with Solution C.
4.3 Gloves
4.4 Compressed gas supply

5.0 PROCEDURE

5.1 Program the appropriate settings on the sprayer.
5.2 Check alignment of spray head with desired line of spray.
5.3 Secure polyester sheet on the platform with magnets.
5.4 Turn on the gas supply and begin spray, watching spray pattern closely for consistency and accuracy.
5.5 Sprayed lines of gold conjugate may be repeated at 1/4" intervals until the sheet is full.
5.6 Dry thoroughly at 37C no longer than overnight.
5.7 Cut into 1/4" strips such that the line of gold conjugate run along the top of each strip.

6.0 CLEAN UP

6.1 Thoroughly rinse the syringe with water, then clean with 1% Triton X-100 (Camag) or 0.02% Bioterge (BioDot), then rinse completely with DI water. Use approximately 10 volumes each of the detergent and the final water rinse.

6.2 Wipe sprayer platform with a damp cloth to remove conjugate residues.

ARISTA BIOLOGICALS, INC.

STANDARD OPERATING PROCEDURE

SOP 04 008 ASSEMBLY OF DIAGNOSTIC TEST CARDS

VERSION NO: 1.0	SUPERSEDES: NEW FORMAT
PREPARED BY: B.SZILAGYI	DATE: 02/23/93
APPROVED BY: J.COLANDUONI	DATE: 02/23/93
REVIEWED BY: B.SZILAGYI	DATE: 09/09/95

1.0 PURPOSE

1.1 To provide guidelines for the assembly of diagnostic test cards.

2.0 SAFETY

2.1 Not applicable.

3.0 EQUIPMENT

3.1 Template

4.0 MATERIALS

4.1 Cards with laminated membrane, sprayed and blocked
4.2 5/8" x 180 mm strips of treated absorbant paper
4.3 3/4" x 180 mm strips of treated absorbant paper
4.4 1/4" strips of sprayed gold conjugate
4.5 gloves

5.0 PROCEDURE

NOTE: The following assembly procedures must be performed under conditions of low humidity, ideally less than 40%. Desiccate top and bottom pads for two hours at 37 C before assembly.

5.1 Remove the liner from the 3/4" glue strip along the bottom of the card. Wear gloves to apply all components.

5.2 Position the gold strip with the line of gold conjugate along the top such that there is approximately a 1 - 1.5 mm overlap onto the membrane.

5.3 Place the 3/4" absorbant pad along the bottom edge of the card, taking care to leave the gold strip exposed.

5.4 Remove the liner from the 1/2" glue strip along the top of the card. Place the 5/8" absorbant strip along the top of the card overlapping onto the membrane by 1 to 1.5 mm.

5.5 Store the finished cards dessicated at room temperature until ready for cutting into test strips. Relative humidity of storage container must be 20% or less.

5.6 If the strips are to be placed into cassettes and pouched, remove only the number of strips from the container that can be placed in fifteen minutes.

CLEAN UP

6.1 Place all unused materials in their original containers.

Name of Assay: ____________ Lot #: ____________ Number of Cards: ______

CONTROL LINE:	TEST LINE:	GOLD LINE:
Reagent: ______ Lot #: ______ Stock Concentration: ______ Working Concentration: ______ Dilution made by: ______ Date ______ Stock Solution: ______ Equilibration: PBS: ______ Batch Volume: ______ Sprayed by: ______ Date ______ Dispenser: ______ Dispense Volume: ______	Reagent: ______ Lot #: ______ Stock Concentration: ______ Working Concentration: ______ Dilution made by: ______ Date ______ Stock Solution: ______ Equilibration: PBS: ______ Batch Volume: ______ Sprayed by: ______ Date ______ Dispenser: ______ Dispense Volume: ______	Reagent: ______ Lot #: ______ Stock OD_{540}: ______ Working OD_{540}: ______ Stablized By: ______ Date ______ Stock Solution: ______ Equilibration: PGB: ______ Sprayed by: ______ Date ______ Dispenser: ______ Dispense Volume: ______

TOP PAD:	MEMBRANE:	POLYESTER SHEET:	BOTTOM PAD:
Lot #: ______ Cut by: ______ Size: ______	Lot #: ______ Laminated by: ______ Block Buffer made by: ______ Treated by: ______ Date ______	Lot #: ______ Solution B made by: ______ Treated by: ______ Date ______ Cut by: ______	Lot #: ______ Solution C made by: ______ PH = ______ Treated by: ______ Date ______ Cut by: ______ Size: ______

CARD ASSEMBLY by: ____________ Date ______
STRIP CUTTING by: ____________ Date ______
DEVICE ASSEMBLY by: ____________ Date ______
DESICCANT Lot #: ____________
DROPPER LOT #: ____________
POUCHING by: ____________ Date ______

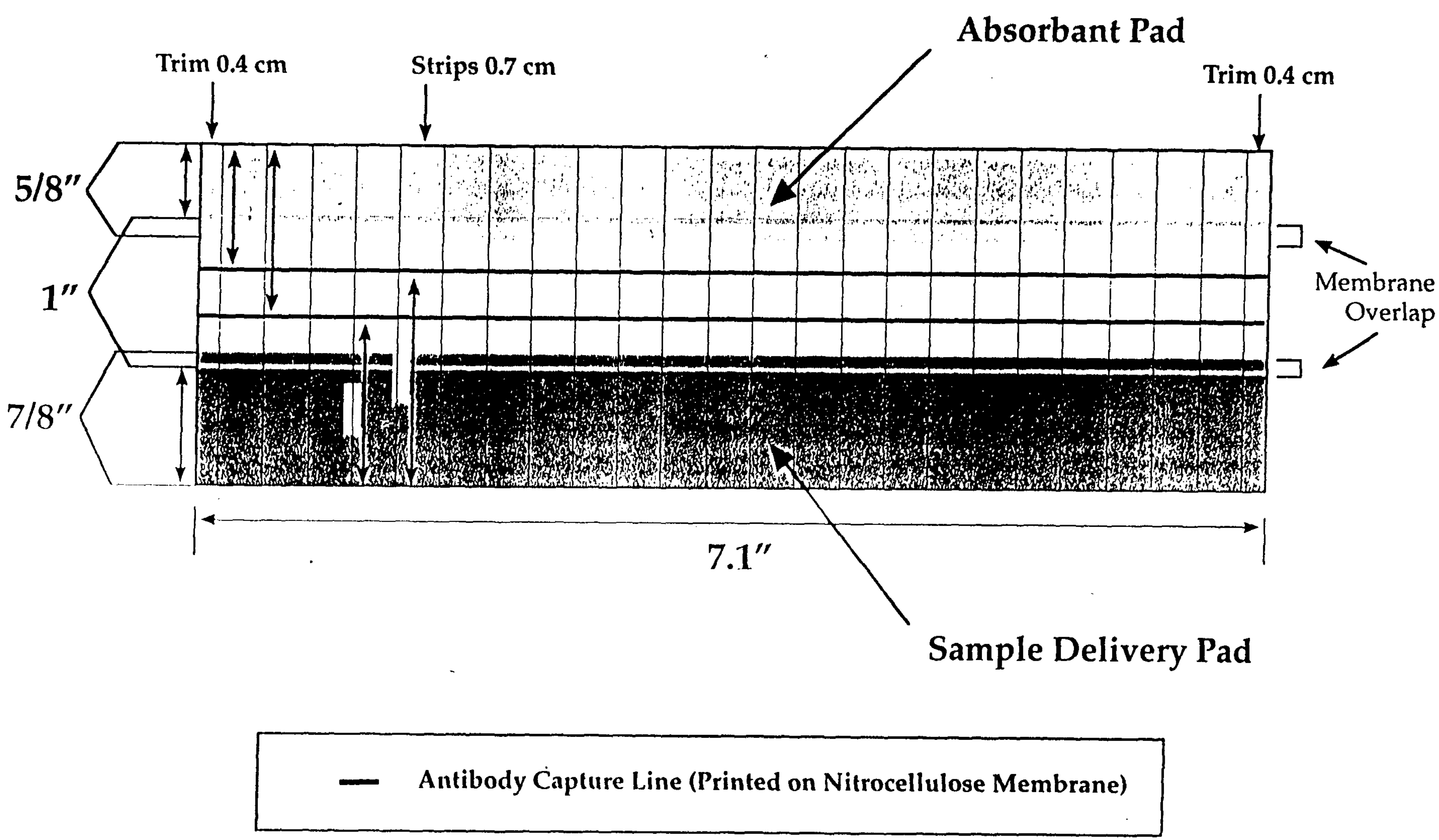
TOTAL NUMBERS OF TESTS: ____________

QUALITY CONTROL by: ____________ DATE: ____________

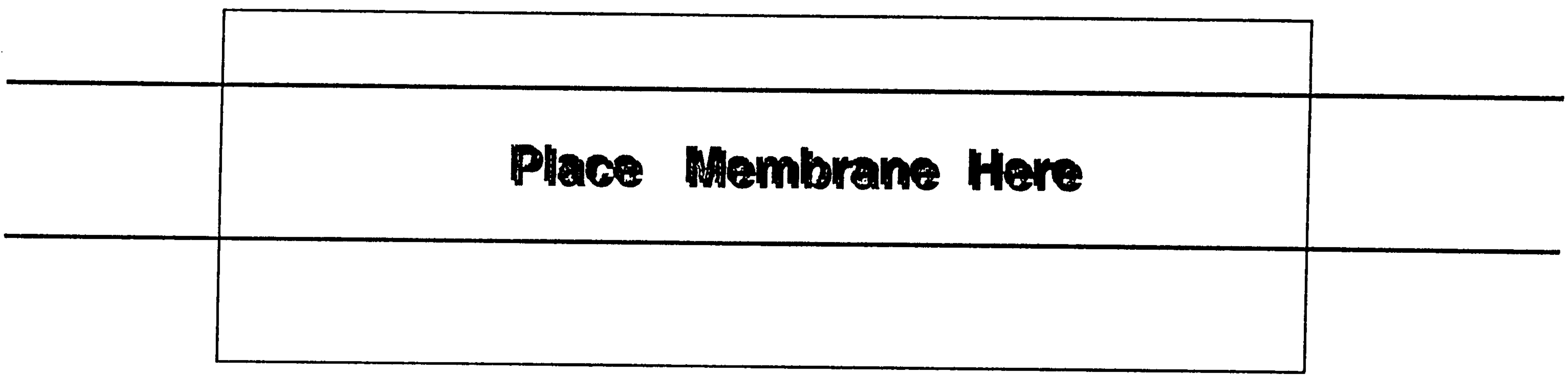
Membrane Placement

Place Membrane Here

Chromatographic Assay Card Template



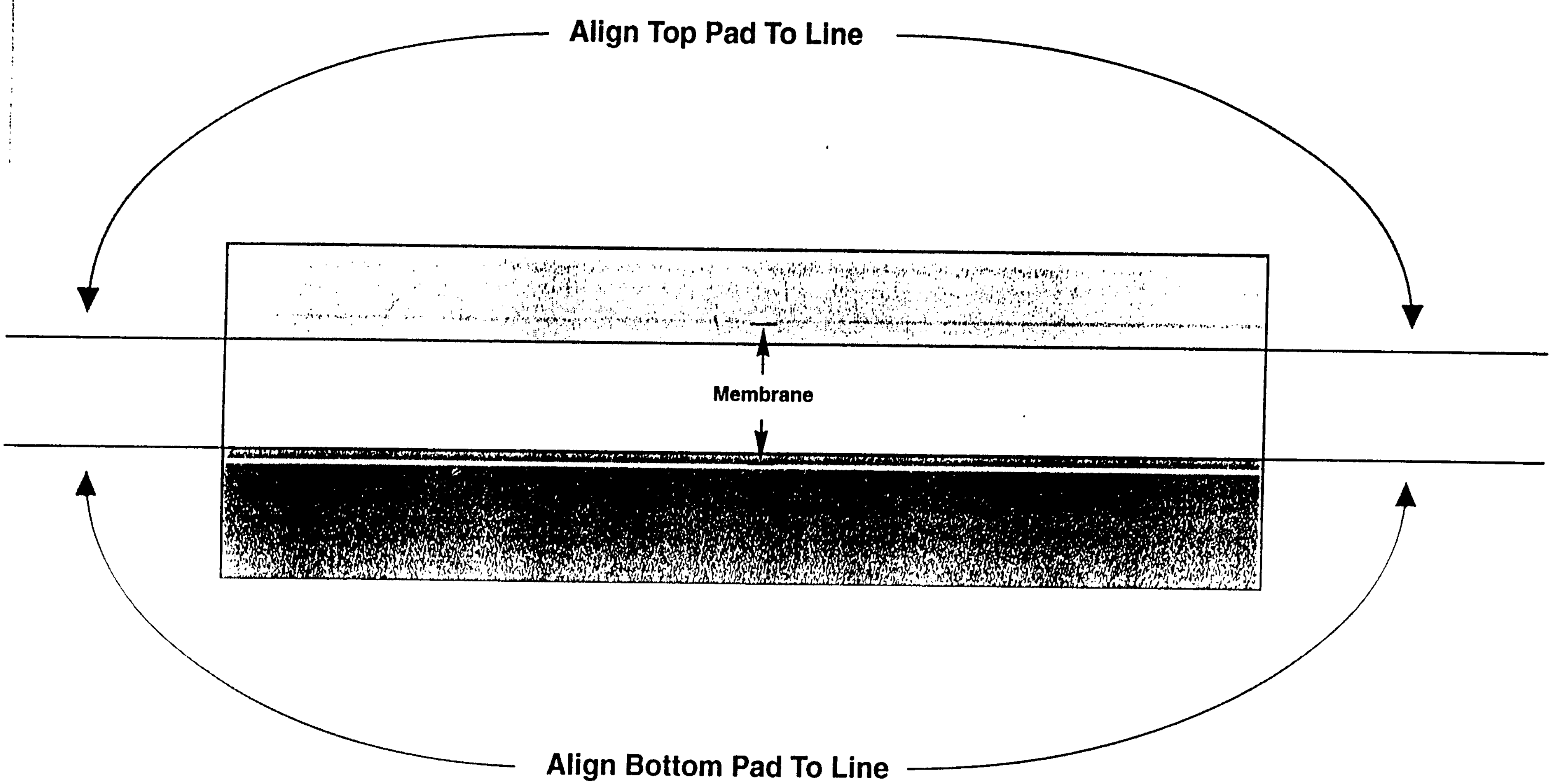
Membrane Placement



Top Absorbant Pad Cutting Template

Bottom Absorbant Pad Cutting Template

Absorbant Pad Placement



ARISTA BIOLOGICALS, INC.
STANDARD OPERATING PROCEDURE AND BATCH RECORD

SOP 06 006 PREPARATION OF PHOSPHATE BUFFERED SALINE (PBS) 10 X

VERSION 1.0	SUPERSEDES: NEW FORMAT
PREPARED BY: B.SZILAGYI	DATE: 10/16/95
APPROVED BY:	DATE:

1.0 PURPOSE

1.1 To provide guidelines and documentaion for the preparation of Phosphate buffered saline (PBS), 10X.

2.0 SAFETY

2.1 Eye and skin protection must be used while adjusting the pH with NaOH 4N.

3.0 BATCH VOLUME__3 liters__PREP DATE________TECH_________

MATERIALS	MANUFACTURER & LOT#	STD AMT (L)	ACT AMT
Sodium Phosphate dibasi		63.9 g	
Sodium chloride		263.1 g	
Deionized Water		QS to 3 L	
HCl 4N			

4.0 PROCEDURE

4.1 Place 2.5 liters DI water into a suitable container with a 3-L capacity.

4.2 Weigh the sodium phosphate dibasic and add to the water with constant stirring until dissolved.

4.3 Weigh the sodium chloride and add to the solution with constant stirring until dissolved.

4.4 Adjust the pH to 7.4 by adding HCl dropwise with constant mixing to the solution.

4.5 Adjust the final volume of the solution to 3 liters with DI water.

5.0 STORAGE LOCATION AND CONDITIONS:

5.1 Location________________________________

5.2 Room temperature

ARISTA BIOLOGICALS, INC.
STANDARD OPERATING PROCEDURE AND BATCH RECORD

SOP 06 007 PREPARATION OF NORMAL PHOSPHATE SALINE FROM 10 X PBS

==

VERSION 1.0 SUPERSEDES: NEW FORMAT
PREPARED BY: B.SZILAGYI DATE: 10/16/95
APPROVED BY: DATE:

==

1.0 PURPOSE

1.1 To provide guidelines and documentation for the preparation of normal phosphate buffered saline from 10X PBS.

2.0 SAFETY

2.1 Not applicable

3.0 BATCH VOLUME____________PREPDATE____________TECH________

MATERIALS	MANUFACTURER & LOT#	STD AMT (L)	ACT AMT
10X PBS		100 mls./ L	
DI water		900 mls.	
NaOH 4N		as necessary	

4.0 PROCEDURE

4.1 Based on batch size calculate the amount of 10X PBS needed. Measure the calculated amount in a graduated cylinder and pour into an adequately sized flask.

4.2 Calculate the volume of DI water needed and pour that measured amount into the flask.

4.3 Stir contents until thoroughly mixed, approximately twenty minutes.

5.0 STORAGE LOCATION AND CONDITIONS:

5.1 Location______________________________

5.2 Store covered at room temperature for up to two weeks. Discard if there is evidence of microbial contamination.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 4 Dec 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Phyllis Rinehart

Encl

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

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